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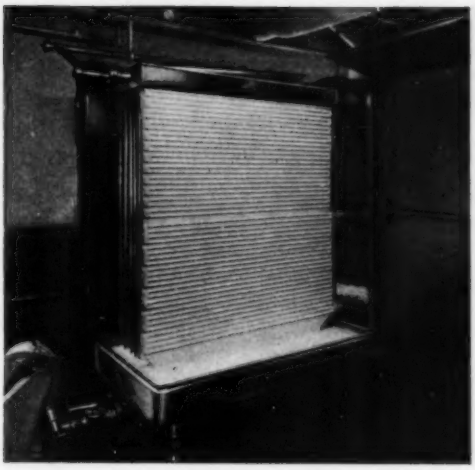

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JOURNAL OF DAIRY SCIENCE

VOLUME XVIII OCTOBER, 1935 NUMBER 10

OBSERVATIONS ON THE FREEZING OF MILK AND CREAM

I. THE EFFECT OF FAT CONCENTRATION UPON THE DISTRIBUTION OF CONSTITUENTS IN THE FROZEN AND UNFROZEN PORTIONS OF PARTIALLY FROZEN MILK AND CREAM*

F. BRUCE BALDWIN, JR. AND F. J. DOAN
Pennsylvania Agricultural Experiment Station, State College, Pa.

FOREWORD

In the freezing of milk and cream pure ice separates from the supercooled liquids in the form of spiny crystals, which, without agitation, usually build up into definite structures. To separate the ice quantitatively from the liquids is almost a physical impossibility since the crystal structures enmesh, by capillarity and obstruction, considerable quantities of the liquids and their components. Consequently, in any separation of the unfrozen and frozen portions of partially frozen milk and cream, short of washing, the frozen portion always contains, in addition to the congealed substance (water), considerable portions of the liquid phase held physically. This is generally recognized and the terms "frozen portion" and "unfrozen portion" as used in this paper are subject to this interpretation. Furthermore, it is realized that milk and cream theoretically would not freeze totally until a temperature lower than the cryohydric point of the ingredient having the lowest cryohydric point is reached. They become apparently totally frozen, however, when no liquid drains from the icy structure and the products are solid masses exhibiting no leakage even when broken up. It is in this sense that the term "total freezing" is commonly used and it is so used in this paper.

It is generally known that as milk freezes the constituents are progressively concentrated in the unfrozen or liquid portion leaving the frozen or

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* Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station, April, 1935, as Technical Paper No. 687. The data presented herein are a portion of those assembled in a thesis by the senior author and submitted in partial fulfillment of the requirements of the degree of Doctor of Philosophy at The Pennsylvania State College.

congealed portion depleted. This was shown as early as 1886 by Veith (1) and has been substantiated numerous times since. A possible exception to this statement might be made relative to the milk fat. The literature exhibits contradictory findings in this respect (2, 3, 4, 5, 6, 7, 8). Some investigators show data indicating that the fat is more concentrated in the unfrozen liquid; others show it to be higher in the frozen portion; and still others obtained contradictory results in different trials. The consensus of opinion seems to be that the degree of freezing, coupled with the time required for freezing (during which interval creaming takes place) influences markedly the distribution of fat between the frozen and unfrozen portions. Henzold (9) found that freezing with mild agitation always produced a higher fat and solids content in the unfrozen portion compared with freezing without agitation. The variations in the distribution of fat and other ingredients between the frozen and unfrozen portions of partially frozen milk and cream have never been systematically studied over the entire range of freezing.

Until quite recently it has been assumed that cream freezes similarly to milk. However, Baldwin and Combs (10) and later Trelogan and Combs (11) found that cream containing 25–30 per cent of fat or over freezes homogeneously so far as the fat is concerned and that an analysis of a sample chipped from the surface of a solidly frozen block will give results checking in per cent of fat with the original unfrozen cream.

The last mentioned workers (12) attribute this homogeneity to the fact that cream of this richness exhibits practically no evidence of fat-rising when aged at low temperatures. This question then arises: Does such cream freeze homogeneously with respect to the other ingredients as well as the fat, and if so, where is the transition point between homogeneous and heterogeneous freezing?

The purpose of the investigation reported here was to study systematically the progressive freezing of milk and cream, over the entire range to total freezing, hoping thereby to explain some of the apparent inconsistencies in the literature relative to fat distribution; to determine whether cream of 25 per cent fat content and over freezes homogeneously with respect to the ingredients other than fat; and, to note the possible effect of the fat phase on the phenomenon.

EXPERIMENTAL

Methods

Skimmilk and whole milk and cream mixtures were standardized to contain 0 per cent, 4 per cent, 13–15 per cent and 25 per cent of fat respectively and were frozen undisturbed in 2-quart covered tin containers in still air at −23 to −28° C. until they had congealed to varying degrees. Upon removal from the freezing chamber the unfrozen portion was immediately

carefully strained through a coarse cheese cloth. Any ice crystals remaining on the cloth were put with the frozen portion and the latter was thawed in a water bath maintained at from 40° to 45° C. until no ice remained. Analyses for the amount of fat, total solids, and titratable acidity, and determinations of the freezing point and the pH were then made on the frozen and unfrozen portions.

Milk fat was determined by the Babcock method, total solids by the Mojonnier method, titratable acidity according to the method of the A. O. A. C. (13), the freezing point using a Hortvet cryoscope (13), and the pH with a Leeds and Northrup potentiometer using the quinhydrone electrode.

The degree of freezing was determined by weighing the frozen portion, and is stated in terms of the percentage by weight of the original.

The results are averages of from three to twelve trials at each percentage frozen for which a point is recorded on the graphs.

In order to make all of the results comparable, the analyses of the original samples (prior to freezing) were used as a reference and the results obtained for the frozen and unfrozen portions are recorded as the differences (either plus or minus) from the original.

Under the conditions used, the time to freeze to 100 per cent (apparently solid) was approximately 12 hours. The time was reduced somewhat as the fat content of the samples increased. The percentage frozen was a linear function of the time up to about 60 per cent frozen after which freezing was less rapid.

Distribution of Fat in Partially Frozen Milk

When fluid milk containing 4 per cent of fat was partially frozen, analyses of the frozen and unfrozen portions made at intervals from the time freezing became evident until almost completely frozen, showed that the fat percentage of the two portions varied considerably and inversely over the entire range of freezing. Figure 1 shows the average results obtained in this study. At the start of freezing the fat percentage of the frozen portions was lower than the original, but after approximately 35 per cent of the fluid was frozen, the fat percentage was higher than the original, and remained higher until approximately 75 per cent was frozen when the difference between the frozen portion and the original became negligible, and remained so until the milk was entirely frozen. The fat content of the unfrozen portion behaved in inverse fashion up to the time about 85 per cent of the milk was frozen after which with further freezing the fat content decreased sharply.

A study of the cross section of a frozen block of milk indicated that creaming of the milk occurred during freezing and suggested that the variable analytical results obtained were probably related to the speed of

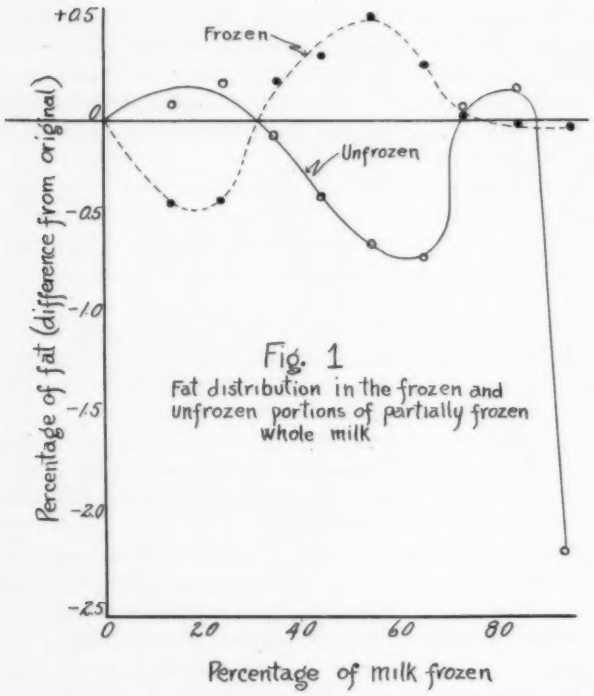
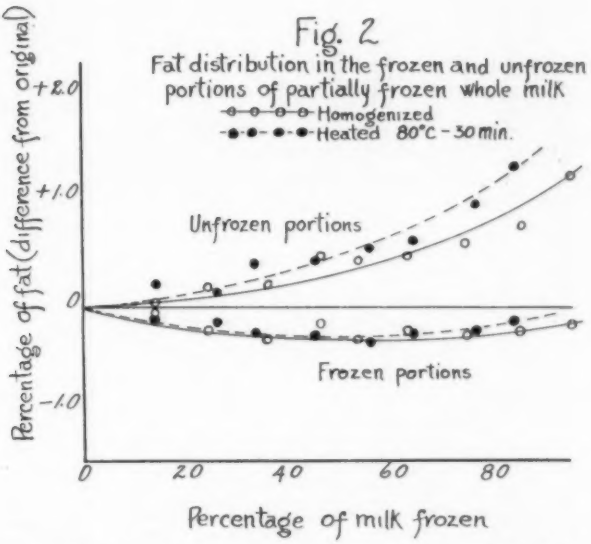


Fig. 1
Fat distribution in the frozen and unfrozen portions of partially frozen whole milk

freezing (particularly of the surface), the rapidity and completeness of creaming and the size and shape of the container. Considering the results obtained, it is not to be wondered at that the data in the literature show such wide diversity.

In order to prove that the analytical results shown in Figure 1 are caused by the creaming phenomenon of the milk, two other series of samples were frozen in similar fashion. These samples were treated to destroy the creaming tendency by homogenization at 2500 pounds pressure and by heating the milk to 80° C. for 30 minutes prior to freezing. The average results obtained in this study are plotted in Figure 2. When the creaming of the milk was prevented by either method, the fat percentage of the unfrozen portion increased progressively over the course of freezing, while that of the frozen portion decreased at first but as the solidly frozen condition approached, the fat percentage increased to nearly that of the original unfrozen milk as would be expected.

These results show that the creaming tendency of milk, frozen without agitation, is the factor responsible for the variable fat distribution in the frozen and unfrozen portions of the product.



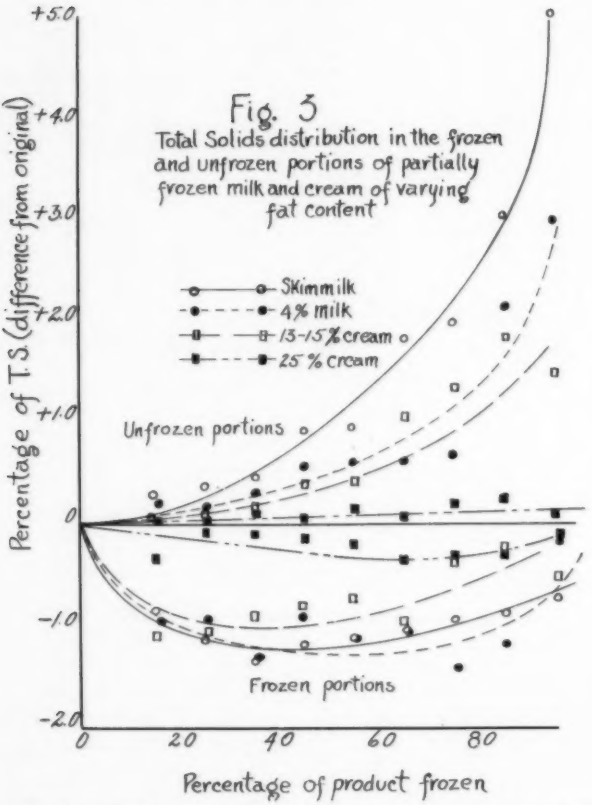
Distribution of Total Solids in Partially Frozen Milk and Cream

It has been shown by other investigators (10), (11), (12), and confirmed again by us that cream containing more than about 25 per cent of fat freezes practically homogeneously so far as the fat is concerned. Whether this also holds true for the other ingredients of such cream, however, has not been previously determined.

The effect of increasing fat concentration upon the distribution of the total solids constituents in the unfrozen and frozen portions of partially frozen milk and cream was studied using skimmilk, 4 per cent milk, 13–15 per cent cream and 25 per cent cream. The average results obtained in several trials are presented in Figure 3. These demonstrate that as the fat concentration of the milk or cream increases, the tendency toward diffusion or concentration of the total solids of milk into the unfrozen portion decreases. The cream containing 25 per cent of fat exhibited almost homogeneous distribution of the total solids throughout the entire course of freezing. It would seem, therefore, that all of the constituents of cream containing more than 25 per cent of fat are distributed homogeneously between the frozen and unfrozen portions of such cream when freezing is allowed to progress without agitation.

Distribution of the Soluble Constituents in Partially Frozen Milk and Cream

It was thought desirable to investigate separately the distribution of soluble ingredients of milk and cream during freezing, since they would be



much more likely to diffuse into the unfrozen portion than any of the others. This was first studied by determining the freezing points of the unfrozen portions of the products used previously. Determinations were made at various degrees of freezing as before. In addition, a fifth series consisting of 13–15 per cent homogenized cream was included in the comparison.

The results are shown in Figure 4. Apparently the fat phase exercises a strong restraining action on the diffusion of soluble substances into the unfrozen portions of the freezing products. This is not particularly evident in the case of 4 per cent milk but shows clearly with the 13–15 per cent cream while with the 25 per cent cream diffusion is apparently prevented entirely. Of particular interest are the results obtained with homogenized 13–15 per cent cream. The creation of a stable fat emulsion and increased viscosity in this product reduced diffusion almost as completely as in the 25 per cent cream. This again emphasizes the relation of cream rising and fat globule

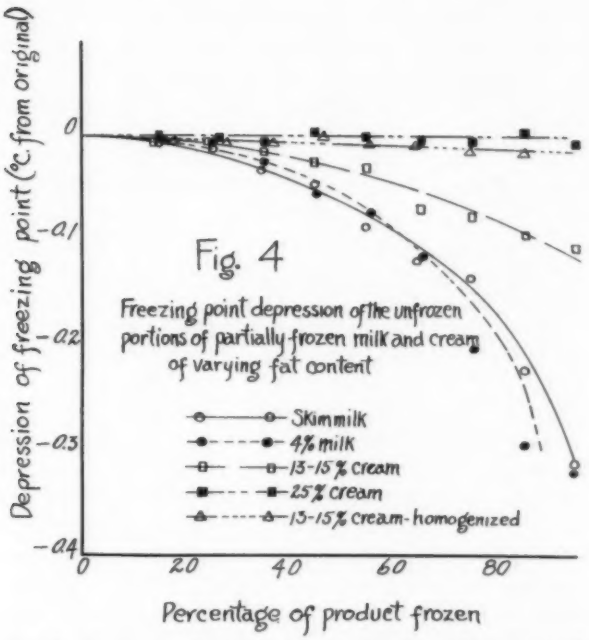


Fig. 4

Freezing point depression of the unfrozen portions of partially frozen milk and cream of varying fat content

clustering to the concentration of materials in the unfrozen portions. Homogenization of the whole milk gave results (not shown) little different from those obtained without homogenization, indicating that the amount of fat is the controlling factor and the stability of the emulsion a secondary one.

Further supporting data showing the effect of the fat phase on the concentration of soluble materials in the unfrozen portions of partially frozen milk and cream are given in Figure 5 and Figure 6. Figure 5 exhibits the pH of the unfrozen portions during the course of freezing and Figure 6 the titratable acidity.

The data show conclusively that no appreciable increase in soluble substances takes place in the unfrozen portion of cream containing as much as 25 per cent of fat in the course of freezing. The data for richer cream are not included. Likewise analyses of the frozen portions of the samples are omitted since the results were what would be expected from a study of the data in Figures 4, 5 and 6.

The slight increase in pH of the unfrozen portion of 25 per cent cream as freezing progress is interesting. In a paper to follow, further data show this occurs consistently when cream is frozen or partially churned, whereas when the emulsion is completely broken, the pH of the serum drops back to about the original figure.

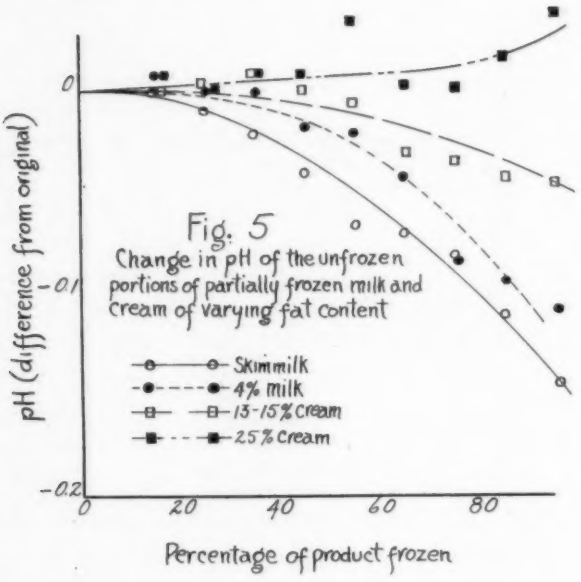


Fig. 5
Change in pH of the unfrozen portions of partially frozen milk and cream of varying fat content

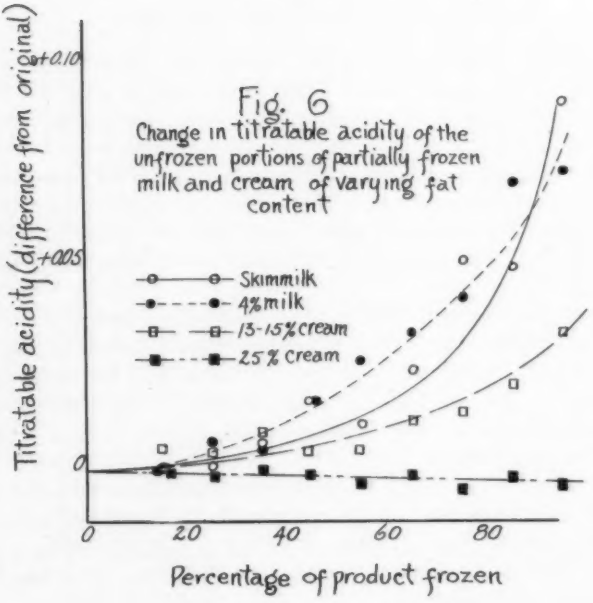


Fig. 6
Change in titratable acidity of the unfrozen portions of partially frozen milk and cream of varying fat content

The decrease in titratable acidity of the unfrozen portion of 25 per cent cream as freezing progresses is to small to be significant and is probably due to the occlusion of small amounts of "acid" substances in the butter particles commonly noted in cream frozen more than 40–50 per cent.

SUMMARY AND CONCLUSIONS

When whole milk is partially frozen in an undisturbed condition, the fat concentration in the frozen and unfrozen portions over the entire range of freezing is dependent upon the cream rising phenomenon of the milk and its speed relative to the speed of freezing; also to some extent, naturally, on the shape and size of the container. Milk that exhibits normal creaming ability shows variable concentrations of fat in the frozen and unfrozen portions from the time freezing first becomes evident until the milk is completely frozen.

When the creaming ability of the milk is destroyed (by heating or homogenization), the fat concentration of the unfrozen portion increases progressively with the degree of freezing, while that of the frozen portion is decreased at first, but finally approaches the fat percentage of the original milk as the degree of freezing approaches 100 per cent.

Increasing fat concentrations in milk or cream retard the diffusion or concentration of the milk constituents (both colloidal and soluble) into the unfrozen portion of the freezing products, and when the fat concentration reaches 25 per cent, such diffusion is practically prevented and the cream freezes homogeneously. The diffusion is possibly inhibited because of the increased viscosity with increasing fat content and also because of the more effective sealing of the interstices between the developing ice crystals by the increased amount of fat in the form of solidified globules.

Homogenization acts similarly to increasing the fat content in restricting the concentration of ingredients in the unfrozen portions of freezing cream provided there is sufficient fat present to give an increase in viscosity.

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VITAMIN A CONTENT OF PASTURE PLANTS

IV. WHITE BLOSSOM SWEET CLOVER (*MELILOTUS ALBA* DESVX.), ORCHARD GRASS (*DACTYLIS GLOMERATA* L.) AND MEADOW FESCUE (*FESTUCA ELATOIR* L.) UNDER PASTURAGE CONDITIONS AND FED GREEN*

ELLA WOODS, F. W. ATKESON, I. W. SLATER, C. D. ARNDT AND R. F. JOHNSON
Idaho Agricultural Experiment Station

In the three previous papers from this station the vitamin A activity of the following six pasture plants were reported: white clover, Kentucky bluegrass, timothy, red top, alfalfa, and smooth brome. The plants reported herein are sweet clover, orchard grass, and meadow fescue. These nine pasture plants are probably more universally used in pasture mixtures than any other plants. Although alfalfa is included in the list it is not used extensively as a pasture crop. Its vitamin A activity in the fresh green state is of interest, however, for comparison with reports on the vitamin A activity of alfalfa hay.

Because of previous literature reviews, citations will be limited to those pertinent to this report. The orchard grass and meadow fescue studied were grown under irrigation at the Caldwell Substation of the Idaho Agricultural Experiment Station. In addition to second-year sweet clover under the same conditions, both first-year and second-year non-irrigated sweet clover grown at Moscow were studied. The same experimental procedure was used as in the second report (6). Fresh green samples of the plants were received at the laboratory twice a week. The samples were obtained under typical pasturage conditions. The period covered by the experimental feeding was later in the summer than the period represented in previous reports on other plants.

RESULTS

The average weight of the rats in all groups was 39 grams at the beginning of the depletion period and 96 grams at the end. The depletion period for all the rats averaged 29 days.

The average growth response of the rats when fed each of the plants as a vitamin A supplement is shown in Table I and Figure 1. Results are presented for periods of four weeks (28 days) and the vitamin A values are expressed in rat units calculated from the dose in milligrams which caused a gain of 12 grams in four weeks.

* Published with the approval of the Director as Research Paper No. 138 of the Idaho Agricultural Experiment Station.

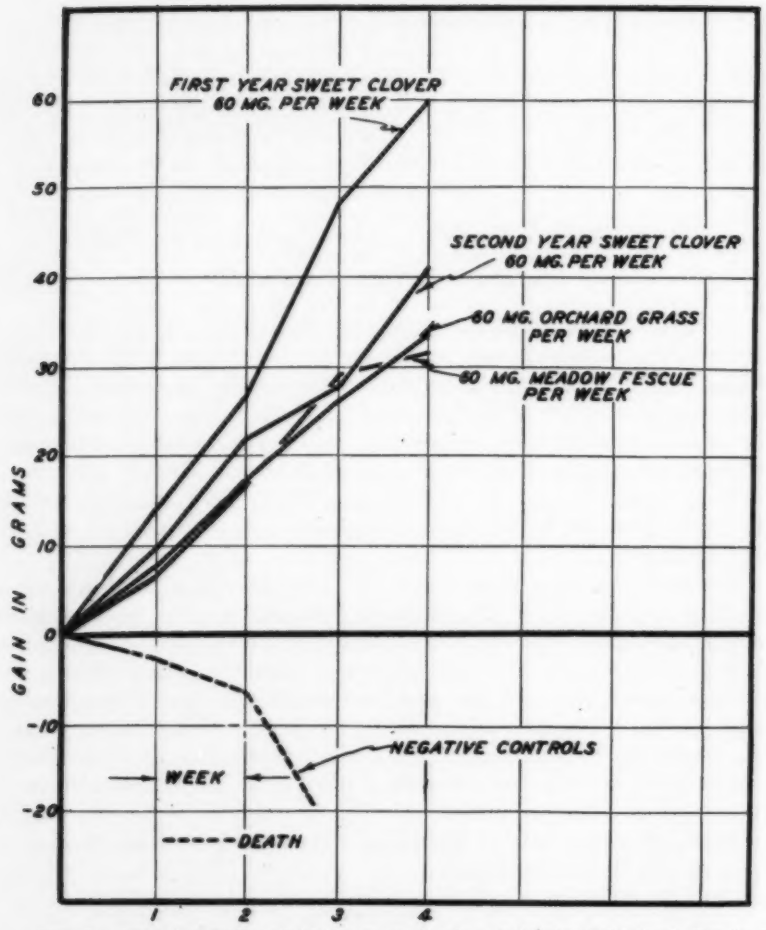

FIG. 1. Average weekly weight increases of rats, previously depleted of vitamin A, when fed either white blossom sweet clover, orchard grass, or meadow fescue as a supplement to a vitamin A free diet.

Average weekly losses in weight of negative control rats were computed each week by averaging the weight of all living rats as long as 50 per cent of the original group survived. The line was terminated by extending it directly from the last week showing 50 per cent survival to a point indicating the average survival days and average weight at death for the entire group. The broken line indicates that one or more deaths occurred during the week.

Based on the growth response obtained with other plants at various levels, each pasture plant in this report was fed at a level of 60 milligrams per week. The animals (10 rats) receiving second-year sweet clover made an average total gain in four weeks of 29 ± 2 grams, which would indicate that this plant contained 242 ± 19 rats units of vitamin A activity per gram. Eleven rats fed first-year sweet clover made an average gain of 60 ± 4 grams, indicating at least 500 ± 30 rat units of vitamin A activity. The Pharmacopoeia of the United States (3), however, prescribes that, in establishing the vitamin A potency of cod liver oil, data shall be considered valid when two-thirds of the animals in a group have made individual gains in four weeks of 12 grams or more and not in excess of 60 grams. Since 5 of the 11 rats fed first-year sweet clover gained more than 60 grams and none less than 37 grams, it would seem that definite evaluation of the vitamin A activity of this plant can not be made without additional feeding tests at lower levels. Comparison of pasture plants on the same feeding level has some advantage in attempting to establish their relative rank in vitamin A activity, and the results are presented for the first-year sweet clover group with due regard for their limitations.

Seven rats that received orchard grass as a vitamin A supplement in 1932 made an average total gain of 33 grams, while seven rats receiving the same grass in 1934 averaged 32 grams gain. The weighted average of the 14 rats in these two groups showed an average total gain of 32 ± 2 grams, indicating that orchard grass contained 275 ± 13 rat units of vitamin A activity per gram.

Meadow fescue was also bio-assayed for vitamin A activity during two different years, 1932 and 1934. In 1932 eight rats averaged 34 grams in total gain and in 1934 four rats averaged 26 grams. The weighted average of the 12 rats was 30 ± 2 grams of total gain in four weeks. This would indicate that the meadow fescue contained 250 ± 13 rat units of vitamin A activity.

The coefficient of variation in the group fed second-year sweet clover was 36 per cent, for the group fed first-year sweet clover 28 per cent, for the orchard grass group 25 per cent, and for the meadow fescue group 27 per cent. The variation in each group compares favorably with what may be expected as reported by Sherman and Burtis (4). Statistical analysis of the results showed that a true difference existed between first-year sweet clover and the other three pastures, the difference being from 11 to 15 times the probable error of the difference. The differences among the vitamin A values of orchard grass (275 rat units), meadow fescue (258), and second-year sweet clover (242), however, were not statistically significant.

In order to be able to compare the results with data reported on the basis of 24 grams gain in 8 weeks as a standard unit of growth, the average total gains for each group of rats for 8 weeks are given. Since rats which die

during the feeding period are often included in the average for a group, such rats were also included in summarizing the data, herein reported, on an 8 weeks' basis.

When the rats were fed 60 milligrams of second-year sweet clover, the total gain in 8 weeks averaged 33 ± 3 grams per rat, indicating the content of vitamin A activity to be 138 ± 14 rat units per gram. Rats fed the same amount of first-year sweet clover averaged 99 ± 6 grams in total gain, indicating that this pasture plant contained 413 ± 27 rat units of vitamin A activity per gram.

Orchard grass fed on the same level resulted in an average total gain of 29 ± 3 grams per rat. This would indicate that the orchard grass contained 121 ± 13 rat units of vitamin A activity per gram. Meadow fescue fed in like quantities caused an average gain of 23 ± 5 grams per rat, or 96 ± 21 rat units of vitamin A activity per gram for this grass.

The rats used as negative controls all showed typical symptoms of vitamin A deficiency at death. Of the 14 rats, 10 had infected sinuses, 10 had infected inner ears, 9 had infection at the base of the tongue, 5 had hemorrhagic intestines, and 5 had infected lymph glands. The negative controls survived an average of 19 days and lost an average of 19 grams in weight.

DISCUSSION

It is interesting to note that first-year sweet clover contained at least twice as much vitamin A activity as second-year sweet clover. This may be accounted for by the fact that first-year sweet clover is much finer stemmed and usually has a larger percentage of leaves than second-year sweet clover. Hauge (1) has reported that in alfalfa hay the vitamin A activity is primarily in the leaves.

In comparison with other pasture plants previously studied (5, 6, 7) the plants herein reported would have the following rank in rat units of vitamin A activity per gram:

First-year sweet clover	500 ± 30
Smooth brome grass	396 ± 27
Red top	308 ± 10
Orchard grass	275 ± 13
Alfalfa	269 ± 17
Meadow fescue	250 ± 13
Second-year sweet clover	242 ± 19
White clover	242 ± 19
Timothy	220 ± 13
Kentucky bluegrass	175 ± 11

In each of the four papers of the series statistically significant differences were found among the grasses studied. For example, red top was statis-

tically superior to timothy in paper II and smooth brome was superior to alfalfa in paper III. By chance, rather widely varying plants were paired for study in the different feeding tests. When the results from all the pasture plants were analyzed statistically, however, different deductions were made.

Considering the pasture plants in the order listed above the difference between any one plant and the one ranking next above or below it was of little, if any, statistical significance. In fact, orchard grass, alfalfa, meadow fescue, second-year sweet clover, and white clover had no significant differences from each other. Selected plants, however, do have significant differences. For example, the difference between red top and timothy is eight times the probable error of the difference.

These facts would indicate that the relative ranking of the plants is justified but that the difference between any adjoining plants as ranked in the list is not particularly significant.

Since the plants studied are among the most universally used pasture plants it would appear that pasture is an extremely potent source of vitamin A activity.

A cow can easily consume 100 pounds of pasture grass daily. If the pasture had a vitamin A value of 200 rat units per gram the total intake of vitamin A activity would be more than nine million rat units.

Moore (2) has shown that the cow's daily output of carotene and vitamin A in the milk fat is very small compared with the carotene intake, never exceeding that of normal summer butter regardless of the amount in the feeds consumed. Considering the large quantity of pasture consumed daily by the dairy cow, and the fact that all the pasture plants have high vitamin A values, it would seem that dairymen might use mixtures of any of the pasture plants adaptable to their particular climatic and soil conditions without the vitamin A activity of the pasture being a limiting factor.

CONCLUSIONS

Biological assay of second-year white blossom sweet clover (*Melilotus alba* Desvx.) indicated that it contained 242 ± 19 rat units of vitamin A activity per gram when sampled under pasturage conditions and fed in the fresh green state. First-year white blossom sweet clover contained 500 ± 30 units per gram. Under similar conditions orchard grass (*Dactylis glomerata* L.) contained 275 ± 13 units, and meadow fescue (*Festuca elatior* L.) 250 ± 13.

The relative rank of these pasture plants compared with others previously reported is as follows: First-year sweet clover, 500 ± 30 rat units per gram; smooth brome, 396 ± 27; red top, 308 ± 10; orchard grass, 275 ± 13; alfalfa, 269 ± 17; meadow fescue, 250 ± 13; second-year sweet clover and

Average Growth Response of Rats when Fed Fresh White Blossom Sweet Clover, Orchard Grass, and Meadow Fescue

PASTURE PLANT	SOURCE	DATE OF TEST PERIOD	AMT. OF PLANT FED WEEKLY	RATS FED	RATS USED		AVE. WT. AT BEGINNING OF DEPLETION PERIOD	AVE. WT. AT BEGINNING OF TEST PERIOD	AVE. WT. AT END OF 4 WEEKS TEST PERIOD	AVE. GAIN IN WT. IN 4 WEEKS	COEFFICIENT OF VARIATION	RAT UNITS OF VITAMIN A
			Grams	No.	No.	%	Grams	Grams	Grams	Grams	%	
(2nd yr.) White Blossom Sweet Clover	Caldwell	7–9–32 to 9–3–32	0.060	8	8	100	42	100	131	31		
"	Moscow	6–3–34 to 7–29–34	0.060	3	2	67	41	93	116	23		
Average				11	10	91	42	99	128	29 ± 2	36	242 ± 19
1st yr.) Sweet Clover	Moscow	7–23–34 to 9–17–34	0.060	11	11	100	41	95	155	60 ± 4	28	500 ± 30
Orchard Grass	Caldwell	7–9–32 to 9–3–32	0.060	8	7	88	39	97	130	33		
"	"	5–30–34 to 7–25–34	0.060	7	7	100	37	97	129	32		
Average				15	14	93	38	97	130	33 ± 2	25	275 ± 13
Meadow Fescue	Caldwell	7–9–32 to 9–3–32	0.060	8	8	100	39	99	133	34		
"	"	5–30–34 to 7–25–34	0.060	5	5	100	38	103	129	26		
Average				13	13	100	39	101	131	30 ± 2	27	250 ± 13
Negative				14			39	92	Average survival—19 days Ave. loss in wt.—19 grams			

white clover, 242 ± 19; timothy, 220 ± 13; and Kentucky bluegrass, 175 ± 11.

Although the differences among the higher ranking plants and the lower ranking ones were statistically significant, little, if any, significant difference existed between any one plant and the plant listed next above or below it.

The pasture plants studied are among the most common plants used in pasture mixtures. The fact that they were all found to have high vitamin A values would seem to justify the conclusion that pasture is a potent source of vitamin A activity.

Considering the large quantity of pasture consumed daily by the dairy cow, and the fact that all the pasture plants have high vitamin A values, it would seem that dairymen might use mixtures of any of the pasture plants adaptable to their particular climatic and soil conditions without the vitamin A activity of the pasture being a limiting factor.

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BACTERIOLOGICAL METHODS FOR THE ANALYSIS OF DAIRY PRODUCTS

P. A. DOWNS, CHAIRMAN, B. W. HAMMER, W. A. CORDES
Subcommittee on Bacteriological Methods for Analysis of Condensed and Evaporated Milk

H. MACY, CHAIRMAN
Committee on Bacteriological Methods for the Analysis of Milk and Dairy Products

FOREWORD

"In 1927, the Committee on Bacteriological Methods of the American Dairy Science Association began a series of reports dealing with the various laboratory procedures suitable for the bacteriological analysis of dairy products. This committee has functioned through the agency of subcommittees, composed of those who are especially interested in certain phases of the dairy industry."

"The objective of these committees has been to make available in condensed form various methods for the analysis of dairy products. The views expressed in these reports are those of Committees appointed by the American Dairy Science Association. As such they are printed for the criticism of other members of the Association and other interested persons."*

THE BACTERIOLOGICAL EXAMINATION OF EVAPORATED MILK

(*Sterilized*)

Evaporated milk, as found upon the market, is primarily confined to cans in which the product has been subjected to sterilization by heat. The usual bacterial content of such milk is relatively low and for that reason the bacteriological study is somewhat different than the study of some of the related products.

METHODS OF SAMPLING

It is extremely important in the sampling of this product, especially if it is taken from the original can, that no contamination occur during handling and that the sample container be sterile. All the utensils that are used or in any way come in contact with the product must be sterile, and the room in which the work is carried out should be as free as possible from atmospheric contamination. This is necessary because of the almost sterile condition of the normal product when in the container.

A very suitable method of handling canned milk previous to sampling is to shake the can and its contents thoroughly, clean the outside of the can, wipe dry with a clean towel and flame the surface to be punctured. In cases where it is desirable to seal the can after removing the sample, the surface

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* See JOURNAL OF DAIRY SCIENCE 16: 277. 1933.

can be tinned with soldering iron before being punctured. This procedure will materially aid in the sealing process. The can-opening appliance should be sterilized in the flame before using. The opening should be only large enough to permit the insertion of a pipette (usually 1 ml.) for the removal of a suitable quantity of the contents, and this should be done as rapidly as possible to eliminate possible contamination from the air. The opening may be protected by covering the entire can with a sterile petri plate half.

If further work is to be carried out with the sample, the contents of the container may be transferred to a glass-stoppered sterile bottle and stored at refrigeration temperatures. The glassware, sampling tubes, etc., should be sterilized in accordance with "Standard Methods of Milk Analysis." If samples are collected in the plant, great care should be taken to see that the product is not in any way contaminated by the movement of the valves on the receptacle from which the sample is drawn. A slight movement of such a valve will often dislodge large numbers of organisms which will be washed out by the product, giving erroneous results. This can be overcome by sampling directly from the large container with some suitable sampling device or if it is absolutely necessary to obtain the sampling through the outlet, it should only be taken after the product has flowed continuously for some few minutes. Either of these methods will help to eliminate inaccurate samples.

If it is necessary to prepare a composite sample it may be secured by combining the various batches, collecting samples as outlined in a container of such a size that it will permit thorough mixing of the product at the time of the analysis.

If the sample of evaporated milk completely fills the container in which it is received, the entire contents should be transferred to another clean, dry, sterile container of ample size to permit proper mixing by shaking. The necessity of uniformity in the sample should be understood by all persons interested in bacteriological work, but the detailed manipulations may be left to the discretion of the individual. It is important during the handling of the products that all possible contamination from a dusty atmosphere or unsterile utensils be prevented. The same general precautions as are applied to the preparation of any sample for bacteriological analysis apply to to the product.

SUGGESTED METHODS OF EXAMINATIONS

(*a*) *Standard Agar Plate Method*

The standard agar plate method (1) of the American Public Health Association, as used for fluid milk, may be used for the examination of evaporated milk.

Media:

Standard nutrient agar prepared according to "Standard Methods of Milk Analysis" (1) may be used.

For special research and investigational work and also routine work involving a study of the possible sterility of the product, it is suggested that beef infusion agar be used. For directions for preparation see Methods for the Microbiological Analysis of Butter (2).

Preparing Dilutions:

It is believed desirable to follow as closely as practical, methods already recognized as standard procedures. For that reason the method of bringing the volume or weight up to 100, as used in the Standard Methods of Milk Analysis of the American Public Health Association (1), is preferred to the method of adding the material to be tested to 10 ml. or grams as the case may be. It is recommended that for normal evaporated milk the volumetric method be used, transferring 1 ml. or 10 ml. of the product to the 99 ml. or 90 ml. water blanks, respectively. Samples that are abnormal physically, such as "curdled" or "lumpy," are best brought to dilution by the gravimetric method.

For direct plating of a low-count milk, one ml. or gram of the sample is placed directly in the petri plate. For a one to ten dilution, it is preferred to measure 10 ml. or weigh 10 grams of the evaporated milk directly into the dilution bottle containing 90 ml. of sterile distilled water. If higher dilutions are desired, they may be prepared from the one to ten dilution or one ml. or gram of the product may be used in 99 ml. of water to obtain a 1 : 100 dilution. The practice of adding the product to water blanks instead of the reverse is suggested because of danger of contamination in transferring a large volume of water; this is important with a low-count or sterile product. Plating should be completed within 15 or 20 minutes after the first dilution in order to prevent growth or death in the dilution bottle which would affect the final results.

In the case of coagulated samples, the use of N/10 LiOH (4) as the diluent in order to aid in getting the product into better solution is advised. In the case of such samples, the diluted product should be kept thoroughly agitated just prior to withdrawal of the sample with the pipette in order that a uniform sample of the solution may be transferred to the petri plate. This condition is most likely to occur in the study of abnormal samples of evaporated milk.

Preparing Plates:

The pouring of the plates should follow the suggestions outlined in the latest edition of the Standard Methods of Milk Analysis (1). With normal evaporated milk, direct plating of the product (1 ml. per plate) will yield either no colonies, or at best, very few. Hence the usual selection of dilu-

tions to yield from 30 to 300 colonies is not possible. However, at least two dilutions should be plated in every case, with duplicate plates for each dilution.

Incubation:

For standard results with standard nutrient agar, use an incubation period of 2 days at 37° C. as recommended in Standard Methods of Milk Analysis (1). Other incubation periods and temperatures may be used for special purposes, such as 5 days at 25° C., 3 days at 30° C., or 2 days at 55° C. The latter temperature is recommended for estimating the number of thermophilic bacteria which may be present.

Counting of Plates:

In the case of those samples which are abnormal and contain material which fails to go into suspension readily, extreme caution should be exercised in counting the incubated plates in order to differentiate between bacterial colonies and masses of the constituents of the samples. Devices for counting the plates with the aid of artificial illumination and low magnification are strongly recommended. Doubtful objects should be examined with higher magnification to determine whether or not they are colonies.

(*b*) *Test for Sterility*

For the purpose of determining whether or not the product in the cans is sterile, it is recommended that sample cans be held at 37° C. for one week. At the expiration of the period the cans should be plated as described above. Cans in which an abnormal physical condition of the milk has developed may have large numbers of organisms present and should be plated in high dilutions. Cans which show the milk in normal condition and which yield no colonies on any of the plates, including 1 ml. direct, may be assumed to be, for all practical purposes, sterile.

(*c*) *Direct Microscopic Count (Breed Method)*

The direct microscopic count (1), as applied to milk, would normally be of little value for evaporated milk because of the low bacterial content of the product, however, in the study of cases of spoilage the method is of value. If it is used for this purpose, it should be carried out according to directions given by Fay (3) for ice cream.

(*d*) *Anaerobic Agar Plate Method*

In the bacteriological study of evaporated milk it is often desirable to obtain information regarding the numbers of anaerobic organisms present in the sample. If this is desired, the same procedure as outlined under "Standard Agar Plate Method" may be carried out, except for the addition of 1 per cent of dextrose to the standard nutrient agar. The methods of

incubation must necessarily be carried out as described in text books dealing with the study of anaerobic bacteria. This method will be of special value in the study of abnormal products and may find a place in certain routine work.

(*e*) *Examination for Thermophilic Bacteria*

For the determination of the presence of thermophilic organisms plates may be prepared from standard nutrient agar. For investigation or research purposes, it is recommended that the following materials be added to each 1,000 ml. of standard agar.

Bacto Tryptophane Broth	2.5 grams
Bacto Yeast Extract	1.0 gram
Dextrose	1.0 gram
Reaction	pH 7.0 ± 0.2

The plates should be incubated for 48 hours at 55° C. At least 15 ml. of medium should be used in each plate to prevent excessive drying during the incubation. Water should be placed in the incubator in order to reduce the drying of the plates to a minimum.

(*f*) *Examination for Spore-Forming Bacteria*

The sample of the product should be placed in a sterile container, heated in a water bath to 80° C. and held for 10 minutes; after cooling, the product should be plated by the standard plate method direct from the sample. Such plates should be incubated 3 days at 37° C. to allow development of such types as *B. coagulans*.

(*g*) *Medium for Special Purposes*

The medium outlined in the standard procedure of the American Public Health Association (1) may not be conducive to the growth of all organisms in average milk. If a special medium is desired, the following is suggested:

Bacto Tryptophane Broth	2.5 grams
Bacto Peptone	2.0 grams
Bacto Yeast Extract	1.0 gram
Dextrose	5.0 grams
Agar	15.0 grams
Water	1000 ml.
Reaction	pH 6.8

Incubation with this medium may be 3 days at 30° C. or any other time or temperature in accordance with the results desired.

METHOD OF REPORTING COUNTS

Results on normal evaporated milk should be reported per milliliter of the product. When the condition of the milk necessitates weighing, results

should be reported per gram. In order to have uniformity in reporting, the medium used and the time and temperature of incubation should always be given in direct conjunction with the numerical results obtained. It is desirable that this procedure be followed when the standard medium and incubation are used as well as in those cases wherein special media and variable incubation conditions are employed.

THE BACTERIOLOGICAL EXAMINATION OF CONDENSED MILK

(*Incompletely Sterilized*)

Under this heading will be included those products that may or may not contain added sugar but which have not been sterilized in the container. The most common of these products is the sweetened condensed milk in hermetically sealed cans. However, much routine bacteriological work is carried out in the examination of sweetened or unsweetened products in bulk containers, such as ten-gallon cans and barrels.

METHOD OF SAMPLING

The same procedure as outlined for evaporated milk may be carried out in sampling products enclosed in hermetically sealed cans. A difference will be found, however, in the type of product. The sweetened condensed milk may be found to be extremely thick and viscous. In such cases it may be advisable to place the can of product in a warm water bath at a temperature not over 37.5° C. for a short time so that the product will become less viscous. In some cases where the separation of sugar has been unusually heavy, it may be necessary to transfer the entire contents of the container to another sterile receptacle to insure a proper mixing. When samples are to be taken from barrels the same precautions should be exercised as outlined above except that it will be impossible to mix the contents. The danger of contaminating the sample when being drawn from the storage tanks should not be overlooked as the movement of valves or pipe joints may dislodge large numbers of organisms which will contaminate the sample and give inaccurate results. If samples must be taken from large masses of the product, they may be obtained by the use of sterile tubes, dippers, or other suitable equipment. Care should be taken that the sample is obtained below the surface which has been exposed to the atmosphere.

If samples are to be taken from barrels they may be obtained either by drawing the sample from the bottom of the barrel or, when taken after the head of the barrel is completely removed, they should be obtained below the surface by some suitable sampling apparatus.

Where it is necessary that composite samples be taken, the final sample should be in a container sufficiently large to allow mixing. Very satisfactory containers for this purpose are glass-stoppered bottles of suitable size.

PREPARING THE SAMPLE FOR EXAMINATION

If the sample of condensed milk completely fills the container in which it is collected or received, the entire contents should be transferred to another clean, dry, sterile container. The detailed manipulations of the process are left to the discretion of the individual. The same general precautions that are applied to any sample for bacteriological analysis should be applied to this product.

SUGGESTED METHODS OF EXAMINATION

(*a*) *Standard Agar Plate Method*

The standard agar plate method (1) as used for fluid milk may be used for determining the numbers of organisms in condensed milk.

Media:

Standard nutrient agar prepared according to "Standard Methods of Milk Analysis" (1) may be used.

For special research and investigational work and also routine work in which maximum counts are desired, it is suggested that beef infusion agar be used. For directions for preparation see Methods for the Microbiological Analysis of Butter (2). The addition of one per cent of sucrose may be desirable in some types of work.

Preparing Dilutions:

For plain condensed (unsweetened) milks it is recommended that the volumetric method be used for the preparation of dilutions. The thicker, sweetened products must be weighed. For the 1 : 10 dilution, measure 10 ml. or weigh 10 grams of the condensed milk into a dilution bottle. In some instances it may be more convenient to prepare the 1 : 10 dilution by using 45 ml. of water and 5 ml. or 5 grams of condensed milk. If a 1 : 100 dilution is desired, this may be prepared from the 1 : 10 dilution, or one ml. or one gram of condensed milk may be diluted with 99 ml. of distilled water. The dilutions may be conveniently prepared by weighing the condensed milk directly into a bottle before adding the dilution water, or if desired, the milk may be weighed into the bottle containing the dilution water. The plating should be completed within 15 to 20 minutes after the addition of water in order to prevent growth or death in the dilution bottle which would affect the final results.

In the case of abnormally thickened milk, it is often desirable to use sterile pieces of broken glass or glass beads in the dilution bottle to assist in the mixing of the sample in the dilution water. In such products the use of N/10 LiOH (4) as the diluent may aid in dissolving the coagulated material.

Preparing Plates: (see page 649)

Incubation: (see page 650)

Counting of Plates: (see page 650)

(*b*) *Microscopic Colony Count* (*Frost Method*)

In laboratories already equipped and experienced in counting bacteria in milk by this method, it would seem that this method could be readily used in the routine control of condensed milk. Directions as outlined in the Standard Methods of Milk Analysis (1) may be followed.

The original sample should be diluted as suggested in the methods for the agar plate count. Incubation should be for 12 to 16 hours at 37° C.

(*c*) *Direct Microscopic Count* (*Breed Method*)

The direct microscopic count (1), as applied to milk, may be of value in the study of condensed milks, especially in unsweetened condensed milks. Where this method is used it should be kept in mind that it cannot be relied upon as an adequate procedure for the determination of bacterial count, but it may be used to give valuable information regarding the product. Where this method is used it should be carried out according to the directions given in the Standard Methods of Milk Analysis (1) or as outlined by Fay (3) for ice cream. It may be of supplementary value in conjunction with certain procedures listed herein.

(*d*) *Examination for Hemolytic Streptococci*

The examination of certain types of condensed milk for hemolytic streptococci may be of value, especially for milk made without the usual hot-well treatment. For this examination, standard nutrient agar to which has been added 0.5 per cent sodium chloride and 2 to 5 per cent defibrinated blood is suggested for plating. The blood should be added aseptically after the agar is melted and cooled prior to pouring the plates. The plates should be incubated for 2 days at 37° C. Examination should be made at the end of 24 and 48 hours.

Suspicious hemolytic colonies should be examined by Gram staining to determine the presence of streptococci. Before definite conclusions can be drawn, reference should be made to standard text-books on bacteriology dealing with the recognition and differentiation of hemolytic streptococci.

If conditions permit its use, veal infusion medium will give somewhat better results than the standard nutrient agar for the growth of the hemolytic streptococci. This medium is prepared as follows:

Ground lean veal	500 grams.
Distilled water	1000 ml.

Infuse overnight in a cold room. Strain through cheese cloth by pressing. Make up to the original volume. Bring to boiling with frequent stirring and boil until the infusion is clear and the coagulum brown. Strain through cheese cloth and filter through paper. Adjust reaction to pH 6.8 to 7.0. Add 0.5 per cent Difco peptone and 0.5 per cent sodium chloride. The broth should then be heated in the autoclave for 15 minutes at 20 pounds pressure. This is a higher pressure than that used for the final sterilization and is

employed to avoid precipitates in the finished product. Egg may be added previous to autoclaving if a glass-clear medium is desired. Before the final sterilization, 1.5 per cent agar is added and the medium distributed in flasks. Filter and sterilize at 15 pounds pressure for 15 minutes. Defibrinated blood (rabbit, horse, sheep, etc.) may be added aseptically just prior to plating and after the agar has been melted and cooled to 50° C.

(*e*) *Examination for Thermophilic Bacteria* (see page 651)

(*f*) *Examination for Bacteria Causing Thickening*

For the determination of the presence of bacteria capable of causing thickening in sweetened condensed milk, the following procedure may be followed: The sample of sweetened condensed milk in question should be diluted with sterile water in a glass-stoppered bottle so that the concentration of sucrose in water in the sample will be approximately 40 per cent. For ordinary purposes approximately 90 grams of milk and 10 grams of water will be sufficient. After the sample has been thoroughly mixed by shaking it should be incubated at 30° C. for a period of one month. If organisms capable of causing bacterial thickening are present the diluted milk will become thick and the titratable acidity materially increased. Cultures of the thickening organisms can readily be obtained by plating on nutrient agar and incubating for 2 days at 37° C.

(*g*) *Examination for Gas-Forming Organisms*

For the determination of gas-forming organisms the inoculation of one ml. of the milk into deep agar of the composition indicated on page 651. A layer of sterile agar may also be placed on top of the hardened inoculated agar and the tube incubated at 37° C. for 48 hours.

Tests for Yeasts Capable of Causing "Swells"

Sucrose-fermenting yeasts capable of growth and gas production in high concentrations of sucrose are always a potential source of trouble in the manufacture of sweetened condensed milk. As a routine check for the detection of possible contamination with these types, it is recommended that sample cans be incubated at 37° C. for extended periods. If "swells" develop the milk should be plated on standard nutrient agar (1) (or beef infusion agar) to which 30 per cent sucrose has been added before sterilization.

As a further check on the condition of sweetened condensed milk immediately after manufacture, it is suggested that enrichment in 30 per cent sucrose bouillon can be used, noting the presence or absence of gas production after incubation for 2 days at 37° C., followed by plating on the sucrose agar. The presence of yeasts capable of fermenting this concentration of sucrose with gas production is the signal for a careful study of sources of contamination in the plant, which must include taking samples at various stages in the manufacture and their subjection to the same test. Whether

or not the types isolated are capable of causing "swells" must be determined by direct inoculation into cans of fresh product.

The direct microscopic count on both the fresh product and that which has been incubated for enrichment purposes may be useful in the examination for yeasts. This would of necessity be supplemented by cultural methods to determine the types of yeasts present.

(*h*) *Examination for Yeasts and Molds*

The determination of yeast and mold counts in condensed milk, while not a common practice, can be used quite satisfactorily and should give valuable information. No doubt a more complete study of yeasts and mold content of condensed milk would give much information regarding the sanitary conditions under which the product was produced. For details of the determination see Methods for the Microbiological Analysis of Butter (2).

(*i*) *Medium for Special Purposes* (see page 651)

METHOD OF REPORTING COUNTS

The results should normally be reported per milliliter or gram of product. One variation from standard methods will no doubt have to be included in the study of condensed milk inasmuch as many of the plates will show less than 30 colonies. In this case it is recommended that the actual number of colonies be used as an indication of the count.

In the interests of uniformity of practice and interpretation of data from various sources, the medium used and the time and temperature of incubation should always be reported in direct conjunction with the numerical results obtained. It is desirable that this procedure be followed when the standard medium and incubation are used as well as in those cases wherein special media and variable incubation conditions are used.

CONCLUSION

The views expressed in this report are those of a committee appointed by the American Dairy Science Association. As such they are submitted for the general criticism of members of the American Dairy Science Association and other interested parties.

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AN UNNOTED HEMOLYTIC STREPTOCOCCUS ASSOCIATED WITH MILK PRODUCTS

JAMES M. SHERMAN AND HELEN UPTON WING
Cornell University, Ithaca, New York

Dick and Dick (5) have reported the presence in small numbers of beta hemolytic streptococci in some of the samples of commercial infant food preparations, made from milk, which they have examined. While these workers made no such statement, it was at first inferred by some that these streptococci might have been the cause of an outbreak of gastro-intestinal trouble in a home for children, but this view was not supported by later work (Dick, Dick and Williams, 6). That hemolytic streptococci are not generally present in significant numbers in dried milks and infant foods containing milk powder is indicated by the surveys of Hucker and Hucker (7) and Supplee and Bixby (12), in which a variety of such products was examined.

Through the cooperation of a manufacturer we obtained a few samples of powdered milk and "protein milk" which contained beta hemolytic streptococci, one of these samples coming from the same factory batch which was examined by the Dicks. The presence of hemolytic streptococci in these products is somewhat surprising, since in their manufacture the milk is first pasteurized and then subjected to the drying process at relatively high temperatures.

On detailed study these organisms were found to possess characteristics which would appear to justify the conclusion that they represent a type of streptococcus not heretofore described. Forty cultures were studied, but since they were all isolated from the products of one factory it is likely that they represent closely related strains of one type.

GENERAL CHARACTERISTICS

The morphological, cultural, and staining properties of this organism are those characteristic of the streptococci and need not be detailed here. Occurrence of the cells in pairs and in short and long chains is somewhat variable on different media and varying conditions of growth, as is true of the streptococci in general.

Action on blood

Active hemolysis of the beta type is produced on horse, human, and rabbit bloods. After five years' culture in laboratory media without blood, it was still hemolytic.

Action on litmus milk

Milk is acidulated and curdled. Litmus in milk is not reduced prior to curdling; slow and incomplete reduction takes place after curdling.

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Action on gelatin and casein

It does not liquefy gelatin, nor is there any visible digestion of casein in milk.

Temperature limits of growth

Growth takes place at 10° C. and at 45° C. The maximum temperature for growth is about 50° C. These facts are important, since none of the pathogenic types of hemolytic streptococci, so far as present information goes, is able to grow at 10° C. (Sherman and Albus, 10; Ayers and Mudge, 2). While the maximum growth temperatures of pathogenic streptococci have not been thoroughly studied, none of them has been reported able to grow at 45° C. This was shown by Sherman and Albus (10) for the types obtained from bovine udders, and by Orla-Jensen (9) with a number of pathogenic streptococci from animal and human sources.

Studies of the temperature growth limits of pathogenic streptococci have been sadly neglected. However, repeated tests in this laboratory with a relatively few authentic strains of *Streptococcus pyogenes*, *Streptococcus epidemicus*, and *Streptococcus scarlatinae* have shown that they do not grow at 10° C. nor at 45° C.

In this connection it should be mentioned that the fecal hemolytic *Streptococcus zymogenes* has been rarely found associated with infections, but is scarcely to be considered a pathogenic species in the usual sense. It grows at 10° C. and at 45° C., and in other characteristics is closely related to the fecalis-liquefaciens groups of streptococci.

Thermal death rates

All of the cultures survived a temperature of 62.8° C. (145° F.) for thirty minutes in skimmed milk, while a majority of them withstood thirty minutes at 65.6° C. (150° F.). Since all of the human pathogenic streptococci, as well as those from bovine udders, thus far studied, are killed by exposure to 60° C. (140° F.) for thirty minutes (Ayers, Johnson and Davis, 1, and other investigators), the high thermal resistance of the organism here described is significant.

Limiting hydrogen-ion concentration

In glucose broth pH values of 4.4 to 4.0 are attained. This is in marked contrast to the human hemolytic streptococci which are inhibited in a pH zone between 6.0 and 5.0 (Ayers, Johnson and Davis, 1, and many subsequent investigators).

Fermentation of test substances

The outstanding feature of this organism as revealed by fermentation tests is its failure to ferment sucrose. Only one of the forty cultures was able to ferment this sugar, and that culture caused only a feeble fermenta-

tion. From the recorded literature it appears that in general the beta hemolytic streptococci ferment sucrose.

All of the cultures fermented glucose, maltose, and lactose; none fermented raffinose, inulin, nor glycerol; only five of the forty strains fermented mannitol, while 37 fermented salicin.

Hydrolysis of sodium hippurate

Sodium hippurate is fermented with the production of benzoic acid and glycine. This test, which was introduced by Ayers and Rupp (3), has been found of especial value in the differentiation of hemolytic streptococci of human and bovine origin. While not to be considered infallible, it is perhaps the best single test for this purpose (Brown, Frost and Shaw, 4). Based upon our present knowledge of the streptococci, the fact that this organism ferments sodium hippurate would appear to add strength to our belief that it is not of importance from the standpoint of human health.

Pathogenicity

The organism is not pathogenic for mice or guinea pigs by intraperitoneal injection, nor for rabbits by intravenous injection. Four mice, two guinea pigs, and two rabbits were each inoculated with one cc. of a heavy 24-hour growth of one of the strains. All of the animals remained in perfect health, and when killed three weeks later for autopsy, no lesions were observed and no organisms were recovered in cultures made from the heart, liver, kidney, and spleen. Virulence tests were not conducted with all of the forty strains.

Relation to other species

This organism, for which the name *Streptococcus hemothermophilus* is suggested, differs markedly from the known pathogenic hemolytic streptococci in its minimum and maximum temperatures of growth, thermal death point, limiting pH of growth, failure to ferment sucrose, non-virulence for laboratory animals, and (from human types) in its action on sodium hippurate.

While this organism resembles the hemolytic *Streptococcus zymogenes* in its temperature limits of growth, the two species are sharply differentiated by their respective actions on gelatin, casein, litmus milk, sucrose, raffinose, and glycerol (MacCallum and Hastings, 8; Sherman and Stark, 11).

Streptococcus hemothermophilus may be easily differentiated from all of the well-described species of non-hemolytic streptococci, in each case (with one exception) by four or more characteristics. According to the tests conventionally used in the study of streptococci, it is most closely related to *Streptococcus fecalis*. These two types are, however, totally different in

their actions on blood agar and their reducing abilities in litmus milk, and usually differ in their actions on mannitol.

ADDENDUM

As this work was done some years ago, some of the newer methods of studying streptococci, such as fibrinolytic action and the fermentation of trehalose and sorbitol, were not used. However, since these tests are of value in differentiating human and animal hemolytic streptococci which fail to hydrolyze sodium hippurate and do not reach a low limiting pH in glucose broth, they would not appear to have any obvious pertinence in this connection at the present time.

SUMMARY

A hemolytic streptococcus which is believed to represent a new species is described. This organism differs from the pathogenic species of hemolytic streptococci in its higher maximum temperature of growth, a lower minimum temperature of growth, a higher thermal death point, and a more acid limiting pH of growth. It also differs from the human types in the hydrolysis of sodium hippurate and its failure to ferment sucrose.

Streptococcus hemothermophilus (n. sp.) is suggested as a name.

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THE EFFECT OF SOYBEANS IN THE RATIONS OF DAIRY COWS UPON THE VITAMIN A VALUE OF BUTTER*

J. W. WILBUR, J. H. HILTON AND S. M. HAUGE

Department of Dairy Husbandry and Research Chemical Laboratory, Purdue University Agricultural Experiment Station, Lafayette, Indiana

It is well known that the vitamin A value of milk and butter is dependent upon the feeds fed the cow. In an earlier publication (1), it was shown that it is possible to maintain through the winter period, by practical feeding methods, the production of butter with the same vitamin A value as that produced by the cows when fed on pasture. Although good quality alfalfa hay was found to be effective in maintaining the quality of winter produced butter equal to that of pasture butter, soybean hay was somewhat less effective. No explanation was offered for the slightly lower efficiency of soybean hay as compared with pasture and alfalfa.

Since the soybean hay, which was used in the above experiment was prepared from soybean plants of excellent quality in the medium bean stage, there existed the possibility that the low vitamin A value of the butter produced by cows fed soybean hay, might be attributed to the presence of the soybeans in the hay. There is evidence that soybeans contain materials which may be of toxic nature and whose detrimental effects are eliminated or destroyed by heat. Thus, Osborne and Mendel (2) found cooking improved the feeding value of soybeans for growing rats. Robinson (3) found that hogs responded much more satisfactorily to cooked soybeans than to raw soybeans as a supplement to corn. Shrewsbury, Vestal and Hauge (4) reported that roasted soybeans have a definitely superior nutritive value to raw soybeans for both hogs and rats. Contrary to the response by hogs and rats, Hilton, Wilbur and Hauge (5) found that raw soybeans as a protein supplement to corn, oats and alfalfa hay can be utilized by dairy calves for satisfactory growth.

It therefore seemed desirable to determine what effect, if any, the beans in soybean hay fed to cows might have on the vitamin A value of butter. In order to study this factor, alfalfa hay formed the dry roughage for one group of cows and late cut soybean hay for the second group, while raw soybeans and roasted soybeans were substituted for linseed oilmeal in the grain ration of each group during successive feeding periods. Since it had been found that roasting the soybeans improved their nutritive value for growing rats and hogs, this offered a possibility that roasted soybeans might prove superior to raw soybeans in producing a butter of high vitamin A value.

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EXPERIMENTAL

Four cows of the Jersey and Guernsey breeds were used in this feeding experiment. These cows were divided into two groups of two cows each and fed according to the schedule shown in Table I. Since it has been previously

TABLE 1

Showing the Composition of the Rations Fed the Cows during the Successive Feeding Periods

	LOT 1		LOT 2	
	Roughage	Grain mixture	Roughage	Grain mixture
Period 1 (6 weeks)	Alfalfa Hay Corn Silage (Check Ration)	400 lbs. gr. white corn 200 lbs. gr. oats 200 lbs. linseed oil meal	Soybean Hay Corn Silage	400 lbs. gr. white corn 200 lbs. gr. oats 200 lbs. linseed oil meal
Period 2 (6 weeks)	Alfalfa Hay Corn Silage	400 lbs. gr. white corn 200 lbs. gr. oats 200 lbs. roasted soybeans (ground)	Soybean Hay Corn Silage	400 lbs. gr. white corn 200 lbs. gr. oats 200 lbs. roasted soybeans (ground)
Period 3 (6 weeks)	Alfalfa Hay Corn Silage	400 lbs. gr. white corn 200 lbs. gr. oats 200 lbs. raw soybeans (ground)	Soybean Hay Corn Silage	400 lbs. gr. white corn 200 lbs. gr. oats 200 lbs. raw soybeans (ground)

shown (1) that alfalfa hay and a balanced grain ration with linseed oil meal as the protein supplement will produce butter of high vitamin value, this ration was used as a check in these feeding trials. All cows were in their third or fourth month of lactation. The alfalfa hay fed to Lot 1 was from second cutting, of excellent quality and field cured in the absence of rain. The soybean hay fed to Lot 2 was of excellent quality. This hay was also field cured in the absence of rain. The soybeans fed in the grain rations of both groups in Period 2 were roasted in a special machine at a temperature of 350° F. for a period of 20 minutes.

At the end of each feeding period, composite samples of milk (equal amounts from each cow) from each lot were collected, the cream separated and made into butter. The butter samples were kept in cold storage at 0° F. and portions removed as needed for biological assays.

The vitamin A values of these samples of butter were determined by biological assay, using the method as previously described (1). The results of

these tests are reported in Sherman and Munsell (6) units, a unit being the amount of butter necessary to give a growth response of approximately three grams per week for eight weeks. The results of these assays are given in Chart 1.

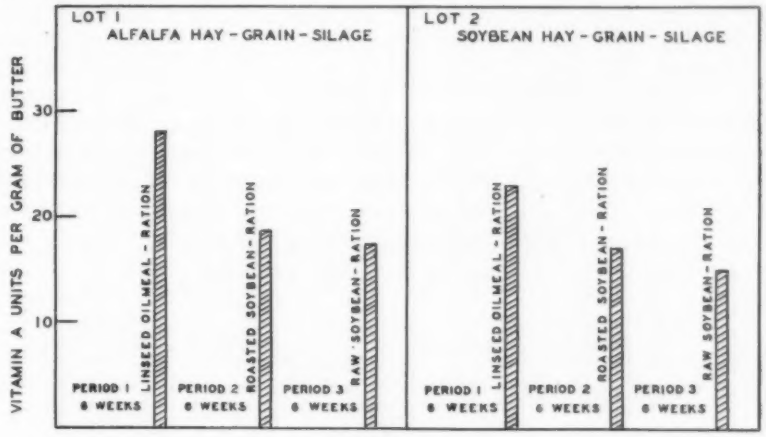

CHART 1.

Showing the suppressing effect of soybeans in the rations of cows upon the Vitamin A value of the butter.

In these experiments, alfalfa hay was the chief source of the vitamin A potency in the rations fed the cows of Lot 1 while soybean hay furnished the vitamin A in Lot 2. The cows of each lot received the same amount of these respective hays each day throughout the successive feeding periods, thus assuring a fairly constant intake of vitamin A, so that any variation in the vitamin A value of the butters produced by these cows during these periods could only be attributed to differences produced by the grain rations. In comparing the biological assays of the butters produced by the cows in Lot 1 which received alfalfa hay as the chief source of roughage, it is to be noted that the substitution of raw soybeans for linseed oil meal in the grain ration resulted in the production of butter with lower vitamin A value. Since the same amount of vitamin A was available to the cows during both periods, the raw soybeans apparently interfered in some manner with the transfer of vitamin A potency from the ration to the butter which resulted in the suppression of the vitamin A value of the butter. The attempt to eliminate the factor responsible for this suppressing action of raw soybeans by roasting the beans, gave such a small increase that it was not considered to be successful. Similar results were obtained with Lot 2.

The suppressing effect of feeding soybeans on the vitamin A content of butter is further emphasized by the relatively lower vitamin A value of the

butters produced by the cows receiving soybean hay (Lot 2) as compared to the cows receiving alfalfa hay (Lot 1). This is particularly noticeable in the first period of each lot where linseed oil meal was used in the grain ration. Apparently the soybeans in the soybean hay were responsible for the marked inhibiting action on the formation of vitamin A in the butter produced by Lot 2. These results are in accordance with our previous work (1).

From the data presented on the vitamin A value of the butter samples produced by the cows fed soybeans, it might appear that the same substance which is apparently toxic to the growth of rats may have been transmitted from the soybeans to the butter. However, this was not the case, because as is shown in Chart 2, the rats gave greater growth response with each cor-

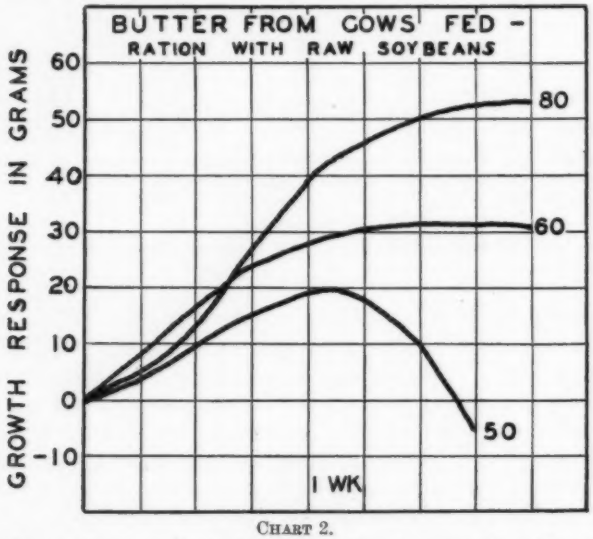

CHART 2.

Composite growth curves of rats showing that there is apparently nothing toxic transferred to the butters produced by cows receiving soybeans in the grain ration.

responding increase in the amount of butter fed. Had there been anything toxic in these samples the higher levels of feeding should have resulted in at least a partial growth failure. Furthermore, the fact that roasting the soybeans, a treatment which improves the nutritive value of soybeans for growth (4), did not eliminate the factor which suppresses the vitamin A value of butter, seems to indicate that the factor responsible for the suppression of the vitamin A value in butter is not the same as that responsible for the repressed growth response in rats.

It should be pointed out that although the feeding of soybeans in grain rations of dairy cattle does have a suppressing effect on the vitamin A forma-

tion in the butterfat, yet butters produced on rations containing soybeans are relatively high in vitamin A value as compared with butter produced by cows fed timothy hay (1) or other roughages of low vitamin A content.

SUMMARY

1. Dairy cows with a constant level of vitamin A intake, produced butter of lower vitamin A value when soybeans were fed in the grain ration than when linseed oil meal was used.

2. Soybeans apparently suppress the transference of vitamin A from the ration to the butter.

3. The attempt to correct this suppressing action by roasting the soybeans used in the grain ration was unsuccessful.

4. Cows fed soybean hay which was harvested after the beans were well formed in the pods, produced butters of somewhat lower vitamin A value than when alfalfa hay was fed.

5. Although soybeans have a suppressing action on the formation of vitamin A in the butterfat, yet it is possible to produce butters of fairly high vitamin A value even when soybeans are used in the rations of the cows provided roughage of high vitamin A potency is fed.

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PREPARING SAMPLES OF BUTTER FOR ANALYSIS

D. H. NELSON
University of California, Davis, California

Preparing samples of butter for analysis is very slow and tedious work mainly because the butter must be softened and thoroughly mixed. The physical and chemical characteristics of butter are such that it is difficult to hasten the softening without causing part of the sample to melt. When melting occurs there are even greater difficulties of properly mixing the sample to a uniform composition. These difficulties are apparent to the carefully trained technician, and usually the proper precautions are taken to avoid serious errors in the preparation of the sample. There are, however, many buttermakers who analyze each churning and technicians who try to speed up their work, that do not avoid melting of the sample around the edges. In such cases more time and care in mixing the sample is necessary in order to produce a sample of uniform composition. In their haste, these analysts may not use the proper precautions to produce a uniformly mixed sample. Mechanical mixers have been employed by some technicians to ensure uniform mixing, but the proper softening of the butter still seems to be important with at least certain types of mixers. In reviewing the literature, the malted milk type of mixer (1) appears to be the only one which has received much attention although modifications of the horizontal spiral bit type have been used successfully for many years.

A new design of the horizontal spiral bit type of mixer has been made to permit mixing samples of various sizes and is suggested as a possible improvement in the apparatus used for preparing samples of butter for analysis. It consists of an electric motor having a speed of 1725 r. p. m. and a horse-power rating of $\frac{1}{4}$ horse power. A chuck similar to that on an electric drill is mounted directly on the shaft. A solid center spiral bit which has had the tip or worm and the two cutters removed, is placed in the chuck. When the motor turns, the bit is turned in the opposite direction from that when it is used for boring. This motion throws the butter into the bottom of the sample jar which is slipped over the bit. The bit should be preferably of $\frac{3}{4}$ inch size and have a solid shaft through the ribs in order to facilitate cleaning. Finally a piece of tin shaped in a half circle is placed on the bench over the bit in order to catch any pieces of butter that may fly off the bit when the sample jar is removed.

Sample jars for use with this type of mixer should be of glass with a tight screw cap, straight sides and flat bottom. The capacity of the sample jar should be approximately 190 per cent of the volume of the sample of butter. The capacity must allow for the volume occupied by the bit. The straight

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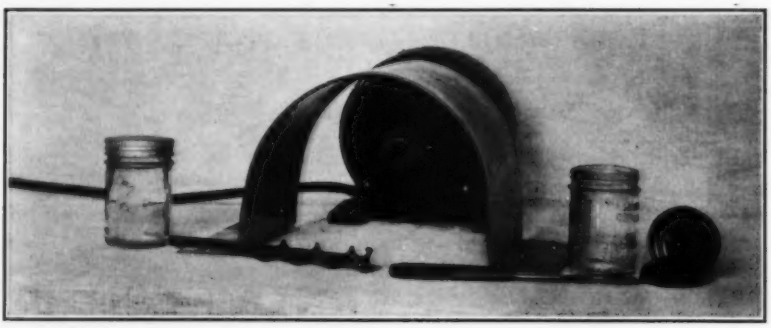

FIG. 1. Horizontal Spiral Bit type of Mixer and Sample Jar. Notice the bit with worm and two cutters removed so that the end is flat.

sides and flat bottom are necessary to permit the bit to reach all corners of the sample jar.

The precautions to observe in using this horizontal spiral bit type of mixer have been established by the following experiments.

SIZE OF MOTOR

Samples of butter at 12° C. were mixed with motors of various sizes. It was found that motors rated below 1/10 horse power did not have sufficient power to maintain the speed when the bit was in the butter and the bearings of the shaft were not capable of carrying the weight of the bit.

EFFICIENCY OF MIXING AT VARIOUS TEMPERATURES

Each sample was held at its respective temperatures 12 to 24 hours to insure a uniform temperature throughout the sample. The sample was

TABLE 1

Maximum difference in 8 tests for per cent total solids of samples mixed two minutes at various temperatures

TIME MIXED IN MINUTES	TEMPERATURE OF SAMPLE		
	12° C.	24° C.	30° C.
2	.22	.24	.24
2	.19	.20	.15
2	.24	.29	.22
2	.31	.28	.43
2	.29	.28	.25
2	.25	.13	.35
2	.19	.26	.29
2	.18	.23	.27
2	.10	.24	.29
2	.16	.22	.36
Average	.21	.24	.29

TABLE 2

Maximum difference in 8 tests for per cent total solids of samples mixed three minutes at various temperatures

TIME MIXED IN MINUTES	TEMPERATURE OF SAMPLE		
	12° C.	24° C.	30° C.
3	.08	.07	.19
3	.09	.11	.23
3	.12	.12	.21
3	.11	.10	.18
3	.09	.09	.20
3	.07	.10	.26
3	.09	.08	.19
3	.08	.07	.21
3	.09	.08	.18
Average	.08	.08	.21

mixed for a definite time as shown in Tables 1 and 2. The prepared sample was then analyzed by the Mojonnier Method for total solids, making one determination from each of eight different parts of the sample. The difference between the highest and the lowest results of these eight determinations on the same sample is called the "maximum difference" in the following tables. Obviously, a sample which is uniformly mixed will have a small "maximum difference."

When mixing these samples it was observed that the sample appeared to be uniformly mixed after from one to one-and-a-half minutes of mixing, especially when the temperature was 24° C. or higher. It was also observed that 30° C. usually caused some of the fat to melt around the sides of the sample jar. This partially separated sample, when carefully mixed, had the same appearance as the samples which were mixed at lower temperatures.

The results in Tables 1 and 2 indicate that the sample must be mixed for some time after it appears to be uniformly mixed. Also when the sample has partially separated, mixing for even three minutes will not make it uniform in composition.

It appears that samples mixed at either 12° C. or 24° C. are uniformly mixed, since the "maximum difference" is within the experimental error of the method of analysis.

Effect of Temperature of Mixing Upon Accuracy

Duplicate samples were prepared from quarter-pound prints of butter by cutting the print into quarters and placing diagonal quarters in a jar for a sample. This gave one-eighth of a pound of butter in each of the duplicate samples. One of the duplicate samples was stored at 12° C. and the other at 24° C. for at least 12 hours in order that the temperature of the entire sample would be uniform.

The samples were mixed for exactly three minutes and then analyzed by

the Mojonnier Method for total solids, making one determination from each of eight different parts of the sample. The average of all eight determinations was assumed to be the correct percentage of total solids of the sample.

TABLE 3

Average per cent total solids of duplicate samples mixed for three minutes at different temperatures

12° C. ORIGINAL SAMPLE	24° C. DUPLICATE SAMPLE	DIFFERENCE ORIGINAL MINUS DUPLICATE
84.66	84.61	+.05
84.63	84.59	+.04
83.94	84.00	−.06
84.27	84.22	+.05
84.65	84.71	−.06
84.81	84.78	+.03
84.69	84.75	−.06
84.83	84.77	+.06
Ave. 84.56	84.55	+.01

None of these samples showed any tendency to melt or separate. The "maximum difference" in the results for any one sample did not exceed 0.11.

CONCLUSIONS

The following precautions must be observed in using the horizontal spiral bit type of mixer:

1. Use glass sample jar with screw cap, straight sides, flat bottom, and a capacity equal to approximately 190 per cent of the volume of the butter sample.
2. Use a motor of not less than 1/10 horse power and 1725 r. p. m., which can be anchored.
3. Do not permit the sample to partially separate as a result of melting around the edges of the sample jar.
4. Mix the sample for at least three full minutes.
5. Stop the motor after mixing each sample and wipe off the one or two grams of butter which adhere to the bit.

This type of mixer has the following advantages:

1. The sample need not be softened or warmed before mixing provided its temperature is between 12° C. and 25° C. This reduces the opportunity to change the composition of the sample. Samples direct from the churn may be mixed.
2. Less time is required for preparing the sample for analysis.
3. It is simple to assemble, operate, and clean.
4. The butter is thrown toward the bottom of the sample jar thus reducing loss and spattering.

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THE THIRTIETH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

R. R. GRAVES
Secretary-Treasurer

The thirtieth annual meeting of the American Dairy Science Association was held at the University Farm, St. Paul, Minnesota, on June 24, 25, 26 and 27, 1935. The program committee, Dr. L. S. Palmer, University of Minnesota, Chairman, arranged programs for the four sections of the association, namely the Manufacturing Section, in which the chief interest is in the dairy products and their manufacture; the Production Section, which is interested in the health, nutrition, breeding and management of dairy cattle; the Extension Section, which is interested in ways and means of presenting educational projects to farmers and the industry; and the Section on Problems and Methods of Instruction, in which the interest is confined to the subject-matter and methods of teaching dairy subjects. The June issue of the JOURNAL OF DAIRY SCIENCE gave the program and in the July issue abstracts of the various papers are given.

The meeting was well attended, as is shown by the following registration by States, prepared by Dr. W. S. Petersen, of the University of Minnesota:

STATE	MEN	WOMEN	CHILDREN	TOTAL
California	3	3	2	8
Colorado	1	—	—	1
Florida	2	2	3	7
Georgia	2	—	—	2
Idaho	1	1	—	2
Illinois	25	7	2	34
Indiana	8	1	—	9
Iowa	15	8	2	25
Kansas	10	4	—	14
Kentucky	5	—	—	5
Louisiana	1	—	—	1
Maryland	3	2	—	5
Massachusetts	4	3	—	7
Michigan	10	1	—	11
Minnesota	102	39	4	145
Missouri	14	5	—	19
Montana	2	1	—	3
Nebraska	8	3	1	12
New Hampshire	4	1	—	5
New Jersey	1	1	—	2
New York	11	5	2	18
North Carolina	3	—	—	3
North Dakota	4	1	—	5
Ohio	15	7	3	25

Oklahoma	5	—	—	5
Pennsylvania	6	2	1	9
Rhode Island	—	—	—	0
South Carolina	6	1	—	7
Tennessee	1	1	—	2
Texas	1	1	—	2
Vermont	1	1	1	3
Virginia	4	—	—	4
Washington	2	1	—	3
West Virginia	1	1	2	4
Wisconsin	25	8	6	39
Wyoming	1	1	3	5
Washington, D. C.	16	7	1	24
Australia	—	1	—	1
Ottawa, Canada	1	—	—	1
Total	325	121	33	479

For a number of years the local committee has arranged programs and trips for the entertainment of the ladies who accompanied the men to the meeting of the American Dairy Science Association. This year one hundred and twenty-one women and thirty-three children registered and were delightfully entertained by the ladies of the local members with the assistance of Minneapolis and St. Paul industries. The program for the ladies follows:

June 24—Monday

8: 30 P. M.—Informal social get-together for members and their families, Home Economics Building, University Farm.

June 25—Tuesday

Hosts for the day—Twin City (St. Paul and Minneapolis) Dairy Industry

9: 00 A. M.—12: 30 P. M. Sight-seeing automobile trip of the Twin Cities.

12: 45 P. M.—Complimentary luncheon, Radisson Inn, Lake Minnetonka.

2: 30 P. M.—Return to University Farm Campus.

4: 00 P. M.—Members and their families leaves for trip to Land-O-Lakes Creameries, Inc.

6: 00 P. M.—Complimentary dinner and entertainment as guests of Land-O-Lakes Creameries, Inc.

June 26—Wednesday

Host for the day—General Mills, Inc.

9: 00 A. M.—Busses will call for the ladies at University Farm and conduct them on a tour of the University of Minnesota Campus, Minneapolis, and of the Washburn Crosby Flour Mills.

12: 00 NOON—Complimentary luncheon at Nicollet Hotel, Minneapolis.

2: 30 P. M.—Return by busses to University Farm, St. Paul.

4:00 P. M.—Tea, Fireplace Room, Home Economics Building, University Farm. Mrs. Walter C. Coffey, Hostess.

5: 00 P. M.—Leave for Minneapolis Automobile Club.

7: 00 P. M.—Subscription banquet and entertainment at Minneapolis Automobile Club.

June 27—Thursday

Shop, golf or other optional activities; or merely rest.

The ladies entertainment committee, that planned and carried out the above program were Mrs. T. W. Gullickson, Chairman, Mrs. Walter Ahlstrom, Mrs. N. N. Allen, Mrs. W. C. Coffey, Mrs. W. B. Combs, Mrs. S. T. Coulter, Mrs. J. B. Fitch, Mrs. E. O. Herreid, Mrs. Harold Macy, Mrs. L. S. Palmer, Mrs. W. E. Petersen, Mrs. B. A. Zakaraisen, Mrs. F. B. Astroth and Mrs. H. R. Searles.

On Tuesday evening the members of the association were the guests of the Land-O-Lakes Creameries, Inc., in an inspection of their large plant and at a dinner at which John Brandt, President of the Land-O-Lakes Creameries, presided. The honor guest was Theophilus L. Haecker, now past 90 years of age, but still alert and active. Professor Haecker was one of the early Professors of Dairy Husbandry in the College of Agriculture of the University of Minnesota. He was a pioneer in the cooperative creamery movement in Minnesota, and was one of the early investigators of the nutritional requirements of farm animals. As a result of his work in the field the Haecker feeding standards were long in use.

On Wednesday noon the association members were the guests of the staff of the University of Minnesota Dairy Department, at a fine luncheon in Haecker Hall.

On Wednesday evening the annual banquet was held at the Minneapolis Automobile Club. The feature of the evening was the presentation of the Association's scroll to Professor Martin Mortensen, Head of the Dairy Industries Department, Iowa State College, as a testimonial of appreciation for his years of outstanding work as a teacher and scientist. Professor E. S. Guthrie, of Cornell University, made the presentation speech. Last year this award was made to Doctor O. F. Hunziker, of Chicago, and the year before to Professor W. F. Fraser, of the University of Illinois. Both of these men were present with their wives and were asked to stand when the scroll was presented to Professor Mortensen. The wording on the scroll follows:

MARTIN MORTENSEN

Friend and Councilor

Professor Martin Mortensen was awarded membership in the Ridder of Dannebrag, the highest honor that the King of Denmark, his native land, can bestow.

Recently he received the honorary degree of Doctor of Laws from Kansas State College of Agriculture and Applied Science, in recognition of his eminent service to the dairy industry.

Two years ago the alumni of his department presented to their alma mater a portrait of their wise and kindly councilor, in honor of his faithful service as Head of the Department of Dairy Industry of the Iowa State College of Agriculture and Mechanic Arts, for a quarter of a century.

In admiration of him and because of his devotion to our Association,

MARTIN MORTENSEN

is given this tribute at the thirtieth annual meeting at St. Paul, Minnesota in nineteen hundred and thirty-five, by the American Dairy Science Association.

R. R. GRAVES, *Secretary* C. L. ROADHOUSE, *President*

The opening session was on Monday evening. President L. D. Coffman, University of Minnesota, and Dean W. C. Coffey, University of Minnesota, were introduced by Professor J. B. Fitch, University of Minnesota, and gave welcoming addresses. Dr. C. L. Roadhouse, President of the American Dairy Science Association, gave a brief address and opened a short business meeting at which were given reports of the Editor of the JOURNAL OF DAIRY SCIENCE, Chairman of the Journal Management Committee, and the Secretary-Treasurer. Following this meeting there was an informal social get-together for members, guests and families at the Home Economics Building.

On Tuesday morning at a joint meeting of the American Dairy Science Association, the Corn Belt Section of the American Society of Agronomy, the American Society of Agronomy, the American Society of Horticultural Science, the Great Planes Section of the American Phytopathological Society, the Genetics Society of America, and American Society of Plant Physiologists, a symposium was held on: "Improving the Germ Plasm of Domestic Plants and Animals. Dr. H. K. Hayes, Chairman, Section O, Vice-president, A. A. A. S.; presided. Dean W. C. Coffey, in an introductory address, stressed the fact that perhaps livestock breeders had paid too much attention to points of the show ring rather than to feeding efficiency and added a plea not to "sacrifice superior germ plasm to the inefficient god of the show." Dr. J. L. Lush's address on "accomplishments with animals" followed. Dr. Lush has recently spent a year in Denmark. Many of his illustrations were taken from data derived from Danish cow-testing associations and Danish pig-recording-testing societies. Dr. O. S. Aamodt, University of Wisconsin, gave an illustrated address on the "Analysis and Synthesis in the Development of New Varieties of Plants." One of the interesting features of Dr. Aamodt's address was his description of "weather machines" in which could be reproduced various combinations of weather, and in which varieties of plants could be tested for their ability to withstand high temperatures and low moisture or their hardiness in withstanding low temperatures. Where, under field conditions, it took about six years to reliably establish facts as to the hardiness of a strain it can now be done with the "weather machine" in one year. The symposium was closed by the reading by Dean Coffey of the following letter from Henry A. Wallace, Secretary of Agriculture:

Dear Dean Coffey:

I am afraid I do not have time to prepare a paper on "Future Possibilities in Plant and Animal Production through Genetic Researches." If, however, the following

would be of any service to you, you can incorporate it into your symposium, if you so desire:

Scientific plant and animal breeding is in its infancy. In the animal world especially, most of the significant results thus far have been obtained by private individuals following empirical methods. These methods, in all too many cases, have had more concern with changing the form and appearance than with changing the functions. The accomplishments of the immediate past are sufficiently great so that we can be confident that the potentialities of the future are rich beyond all imagination. But of very necessity, the time factor is such that the progress in any one year or even five-year period will seem distressingly slow.

As we endeavor to segregate superior strains of plant and animal germ plasm and bring about that combination of superior strains which will result in the most efficient functioning organisms, we must remember that not only must there be a carefully planned scientific approach set up over the years but also there must be a profoundly sympathetic attitude toward the organisms with which the work is being done. Scientific analysis is not enough. There must also be love of synthesis on the part of individuals who have a definite affection for the particular species of plant or animal, the innate character of which is being improved. A true plant and animal breeder must also be something more than a Mendelian geneticist. As a matter of fact, the best plant and animal breeders may be only moderately thorough in their understanding of Mendelian genetics. They may be only moderately good in the fields of statistical comparisons, but they must have that sympathetic understanding of life, that highly developed power of observation which characterized the nature students of the ninteenth century and which should now characterize the plant and animal breeders of the twentieth century as they attempt to improve the functions of the organisms in which they are so intensely interested. If the Universities, Experiment Stations, and the Department of Agriculture can furnish facilities to the various individual workers to continue broad-gauged programs over a long period of years, the results fifty years hence, I am convinced, will be truly amazing.

In closing, I would like to express my belief that, in all probability, there will be an ever-growing intensity of scientific interest in the functions of living forces as contrasted with the more static inorganic, mechanical forces. As this change in human interest gradually comes to pass, I am sure that life will become definitely richer.

PUBLICATION OF ABSTRACTS

O. F. Hunziker reported, as Chairman of the Journal Management Committee, that a plan has been evolved for the Association to publish abstracts of dairy literature. The proposed plan had the unanimous approval of the Board of Directors and after presentation to the convention, was also approved by the membership.

The report of the Secretary-Treasurer was read and approved. The report follows:

THE SECRETARY-TREASURER'S REPORT

Membership

Following is a table showing the membership of the association as of June 15, 1935. The number of new members and associate members that have come in for this year are shown by States together with the number of old members who are in good standing, the total number of members and associate members in good standing by States—and

the number of active members in 1934 who have failed to date to renew their membership for this calendar year.

The totals show that as a result of the campaign for new members this year, 152 new members and 20 associate members have been secured, giving us 640 full members and 21 associate members, or a total of 661 active members to date. This exceeds the total number of active members in 1934 by 39. It may reasonably be expected that the total number of active members for 1935 may go as high as 675.

The unfortunate feature of this showing is that 151 members who were active in 1934 have failed to renew their membership for 1935. These 151 non-renewals constituted close to one-fourth of our 1934 membership.

Our turnover in membership has been high for several years. This year and last we had active and successful campaigns to increase our membership, and the turnover has increased. This failure of so many to renew their membership is probably due in part to economic conditions and in part to the fact that many new members who have joined our association during these membership campaigns have not found in our association or in our Journal what they needed. We are discussing today the possibilities of abstracting the dairy literature for publication in the JOURNAL OF DAIRY SCIENCE. It may be that these abstracts will more nearly meet the needs of many members who are engaged in commercial work than do the technical articles that predominate in the Journal.

We are still far from having the number of members, that the size of our industry warrants, when gauged by the number of men who are interested in or connected with its technical or scientific phases. In January 1930, when I became Secretary, we had about 400 members. The greatest increase in members has come in the past two years, due to the active membership campaigns conducted by Presidents Stoltz and Roadhouse, with the splendid cooperation of members in the various States. If the membership is to be maintained at its present level, or increased, it appears that such membership campaigns will have to become regular yearly affairs.

Some of you who have served on membership committees that have sent in the names of applicants for membership may get the wrong impression from the accompanying table in that new members are credited to the States from which the applicants were recommended. For instance Dr. Ellenberger's Committee in Vermont have sent in a number of applications but the applicants were all residents of States other than Vermont.

1935 Membership in American Dairy Science Association

STATE	NEW MEMBERS		OLD MEMBERS		TOTAL		1934 members not renewed
	Full	Associate	Full	Associate	Full	Associate	
Alabama	1	—	1	—	2	—	—
Arizona	—	—	3	—	3	—	4
Arkansas	—	—	1	—	1	—	1
California	12	1	34	—	46	1	5
Connecticut	1	—	13	—	14	—	4
Washington, D. C.	6	—	17	—	23	—	4
Florida	1	—	4	—	5	—	1
Georgia	—	—	2	—	2	—	1
Idaho	—	—	3	—	3	—	1
Illinois	9	—	39	—	48	—	14
Indiana	1	—	19	—	20	—	4
Iowa	1	—	15	—	16	—	3

Kansas	1	—	8	—	9	—	1
Kentucky	—	—	5	—	5	—	—
Louisiana	—	—	3	—	3	—	1
Maine	—	—	3	—	3	—	—
Maryland	4	—	10	—	14	—	4
Massachusetts	6	—	11	—	17	—	4
Michigan	9	1	17	—	26	1	1
Minnesota	21	—	19	—	40	—	2
Mississippi	—	—	2	—	2	—	1
Missouri	4	—	17	—	21	—	2
Montana	2	1	2	—	4	1	4
Nebraska	3	—	10	—	13	—	2
Nevada	1	—	1	—	2	—	—
New Hampshire	1	—	4	—	5	—	—
New Jersey	4	—	7	—	11	—	4
New Mexico	—	—	2	—	2	—	1
New York	8	—	57	—	65	—	16
North Carolina	2	—	3	—	5	—	1
North Dakota	—	—	3	—	3	—	1
Ohio	7	16	31	—	38	16	15
Oklahoma	2	—	4	—	6	—	1
Oregon	2	—	8	—	10	—	4
Pennsylvania	14	—	27	—	41	—	13
South Carolina	—	—	5	—	5	—	6
South Dakota	1	—	2	—	3	—	1
Tennessee	3	—	2	—	5	—	7
Texas	1	—	3	—	4	—	1
Utah	4	—	3	—	7	—	—
Virginia	3	—	3	—	6	—	2
Vermont	—	—	13	—	13	—	5
Washington	4	—	9	—	13	—	—
West Virginia	1	—	10	—	11	—	3
Wisconsin	5	1	23	1	28	2	—
Wyoming	—	—	1	—	1	—	—
Total	145	20	479	1	624	21	145

Membership in United States Territories and Other Countries

	NEW	OLD	TOTAL	DID NOT RENEW
Canada	5	9	14	3
Denmark	–	2	2	–
France	1	–	1	–
Germany	–	1	1	–
Holland	–	1	1	–
Japan	–	1	1	–
Russia	–	1	1	–
Scotland	–	1	1	–
Porto Rico	–	1	1	–
Panama	–	1	1	1

Alaska	–	–	—	1
Ireland	1	–	1	–
Italy	–	–	—	1
	—	—	—	—
Total Foreign	7	18	25	6

	FULL	ASSOCIATE	FULL	ASSOCIATE	NOT RENEWED
United States	145	20	479	1	145
Foreign	7	—	18	–	6
	—	—	—	—	—
	152	20	488	1	151

Total full members	640
Associate members	21
	—
	661

Finances

The finances of the association are best shown by the balance sheet as of December 31, 1934, and the Profit and Loss Statement for the Period November 27, 1933, to December 31, 1934. These follow, together with an explanatory letter from Charles E. R. Adams, Public Accountant.

10 Knowles Avenue
Kensington, Maryland
February 5, 1935.

Mr. Roy R. Graves
Secretary and Treasurer
American Dairy Science Association
Washington, D. C.

Dear Mr. Graves:

In accordance with your request I have made an examination of the records of the American Dairy Science Association, installed a complete set of double entry books, and submit herewith the following statements and comments:

"EXHIBIT A"—BALANCE SHEET
as at December 31, 1934.

"EXHIBIT B"—STATEMENT OF OPERATIONS
For the period November 27, 1933 to December 31, 1934.

Comments:

Cash in Bank as shown by the books was reconciled with bank statements furnished by the McLachlen Banking Corporation. Cash on hand represents one money order, not included in 1934 deposits.

All records furnished me were recorded on double entry books and all accounts were closed at December 31, 1934.

Bonds appear on the balance sheet at par and were recorded according to your information.

Accounts Receivable—Returned Checks were set up from information obtained from your bank statements. As insufficient records were kept of these items it was impossible to trace their final disposition. It is quite probable that the majority of these checks have been made good and the amounts of same included in various deposits. If this

is the case, membership fees or subscriptions are overstated to that degree. However, future adjustments will clear these items in proper order.

I have estimated the inventory taken by Science Press of Journals on hand at December 31, 1934, to be $525.00. Eighty per cent of this amount has been set up in a reserve to decrease the inventory to a conservative value, future sales of old copies being doubtful.

As there was no inventory of Journals taken at the beginning of the period audited, and the records were incomplete, I was unable to compute the beginning inventory for the purpose of showing the cost of goods sold.

The Profit and Loss Statement shows the amount of purchases as expenses under the various headings. These costs were recorded on a cash basis from the cash book.

Deferred income represents payments made in 1934 on 1935 subscriptions and membership.

In order to facilitate future audits and provide sufficient data for verification of account balances, I recommend the following procedure.

Request your publisher, Science Press, to submit names and addresses of subscribers and advertisers along with their remittances, and record in detail in this report, retail price and discount allowed as well as amount remitted.

Request also a detailed statement of all Journals sold, showing name and address and number of copies of each volume and the same information on advertising contracts. This applies to gratis copies as well.

This information will be of value in the verification of inventory and amounts received.

Respectfully submitted,
CHARLES E. R. ADAMS, *Public Accountant*

AMERICAN DAIRY SCIENCE ASSOCIATION
WASHINGTON, D. C.

"EXHIBIT A"

BALANCE SHEET
as at December 31, 1934.

Assets

Cash in Bank	$3950.40	
Cash—on hand—undeposited	2.00	$3952.40
ACCOUNTS RECEIVABLE—		
Advertising Accounts:		
Creamery Package Mfg. Co.	20.82	
Mitchell Faust	7.00	
Difco Laboratories	13.00	
Returned Checks	35.00	75.82
Inventory—Journals (see comments)	525.00	
Less reserve	420.00	105.00
Investments—Bonds		3000.00
TOTAL ASSETS		$7133.22

Liabilities and Capital

ACCOUNTS PAYABLE	$ 955.88
Deferred Income	398.05

CAPITAL:		
Capital—November 27, 1933 per financial statement of that date	5437.49	
Add: Estimated value of Inventory of Journals at December 31, 1934	105.00	
Net Profit for period, November 27, 1933, to December 31, 1934, transferred from "Exhibit B"	236.80	5779.29
TOTAL LIABILITIES AND CAPITAL		$7133.22

AMERICAN DAIRY SCIENCE ASSOCIATION
WASHINGTON, D. C.

"EXHIBIT B"

STATEMENT OF PROFIT AND LOSS
For the Period November 27, 1933 to December 31, 1934.

INCOME:			
Membership Fees		$3020.30	
Subscriptions		2939.72	
Advertising		1375.45	
Single Copies		9.50	
Back Copies		85.80	
Reprints		83.74	
Sale of Abstracts		43.53	
Sale of Bulletins		50.00	
Miscellaneous Income		2.25	
GROSS OPERATING INCOME			$7610.29
OPERATING EXPENSES:			
Journal		5128.10	
Reprints		150.30	
Handling of advertising & subscriptions		571.97	
Postage & Stationery—Science Press		129.03	
Editorial Expense:			
Salary	$750.00		
Telegrams	1.13		
Postage & Supplies	89.25	840.38	
Presidential Expense		46.50	
Secretary and Treasurer			
Salary	300.00		
Postage	31.00	331.00	
Divisional Allotment		100.00	
Accounting & Bookkeeping		100.00	
Association Stationery		22.00	
Federal Check Tax		1.02	
Collection and Exchange		3.61	
Miscellaneous		26.20	

TOTAL OPERATING EXPENSE		7450.11
NET OPERATING PROFIT		160.18
NON-OPERATING INCOME:		
Bond Interest	112.50	
Bond Premium on U. S. Bonds	35.88	76.62
NET PROFIT TRANSFERRED TO "EXHIBIT A"		236.80

The report of the Auditing Committee also follows:

Mr. R. R. Graves,
Secretary-Treasurer,
American Dairy Science Association,
Bureau of Dairy Industry,
Washington, D. C.

Dear Mr. Graves:

The auditing committee has examined the books and records of the association for the fiscal year November 27, 1933, to December 31, 1934, and finds them to be correct and in good order. Since the financial statement includes the expenses of 13 months, but the income of only 12 months, the profits for the year 1934 are acutally greater than is shown.

The committee recommends that the books be audited annually by a competent public accountant, provided the cost is reasonable (approximately $25.00).

The committee further recommends that an additional $1000 be invested in Government Bonds at this time. It feels that purchase of $2000 worth of bonds at the present time would excessively lower the bank balance which now includes most of the income for the year 1935, but from which the larger part of the year's expenses must still be paid.

Very truly yours,

JOHN I. ORMOND
(for H. F. Judkins)
L. A. ROGERS
B. H. WEBB

At the 1934 annual meeting the Board of Directors authorized the Secretary-Treasurer to change the fiscal year to the calendar year, and to balance the books of the association as of December 31 instead of November 30, as in the past. Consequently the above statement covers a period of 13 months. Some of the operating expenses for the last half of 1933 were paid in December of 1933 and are therefore included in the above statement. The salary of the Editor and of the Secretary-Treasurer as listed on Statement of Profit and Loss covers a year and a half because the salaries for the last half of 1933 were paid in December, 1933. Therefore, the net profit for 1934 is actually nearer $600.00 than the $236.80 given in the statement.

For a comparison of the finances of the association, as given on the Balance Sheet as of December 31, 1934, it may be stated that in January, 1930, the capital of the association was $1,411.17.

The report of the Editor of the JOURNAL OF DAIRY SCIENCE was read and approved. A brief of the Editor's report follows:

The reports of the Editor of the JOURNAL OF DAIRY SCIENCE was received and approved. This report shows that in spite of the monthly publication of the JOURNAL, and its consequent increased expense for the year 1934 as against the bi-monthly publication prior to 1934, the JOURNAL business shows a small profit. This was made possible by the very considerable increase in membership fees resulting from the membership drive that was so successfully conducted during the past year. The report further emphasizes that the increased size of the JOURNAL, the more frequent publication, the increased use of the JOURNAL for Association affairs, and the trend toward an increased proportion of members to subscribers, all indicate an improved JOURNAL and a stronger Association.

The following report of the nominating committee was received and approved:

For Vice-President:

J. R. Dice, North Dakota.

R. R. Graves, Washington, D. C.

For Director for 3-year term:

J. W. Linn, Kansas.

C. R. Gearhart, Pennsylvania.

Submitted:

H. A. BENDIXEN, Washington
R. B. BECKER, Florida
J. M. SHERMAN, New York
H. B. ELLENBERGER, Vermont
E. L. ANTHONY, Michigan, *Chairman*

BUSINESS CONDUCTED BY THE BOARD OF DIRECTORS

Officers and members of the Board present were: Dr. C. L. Roadhouse, President; H. A. Ruehe, Vice-President; R. R. Graves, Secretary-Treasurer; Professor Martin Mortensen and Dr. O. F. Hunziker, members of the Board. Dr. L. A. Rogers, the third member of the Board, was unable to attend the meeting.

The following motions were adopted:

1. To hold the 1936 annual meeting at Pennsylvania State College, on the dates of June 17, 18, and 19, 1936.

2. To adopt the Registration Fee method of defraying expenses incurred in the holding of the annual meeting. The fee was set at $1.00 for members and $2.00 for non-members. Families of members attending the annual meeting will not be required to pay a fee.

3. A vote of thanks was extended to Professor A. A. Borland for the invitation to hold the next Annual Meeting at Pennsylvania State College.

4. The secretary was directed to send the following telegram to Doctor A. C. Dahlberg, Editor of the JOURNAL OF DAIRY SCIENCE. "Directors of American Dairy Science Association express sympathy your illness and regret your inability to attend meeting."

5. The JOURNAL Management Committee was instructed to draw up a new agreement relative to the publishing of abstracts in the JOURNAL, to be submitted to the general meeting for action. (Submitted and approved—see report.)

6. That the JOURNAL Management Committee for the following year be the same as the past year, that is, Dr. O. F. Hunziker, Chairman, Professor A. A. Borland, and the Secretary.

7. The appointment of Professor R. B. Stoltz, of Ohio State University, as Secretary-Treasurer for the next fiscal year—effective January 1, 1936.

8. The reappointment of Dr. A. C. Dahlberg as Editor of the JOURNAL OF DAIRY SCIENCE for the coming year.

At the meeting of the association on June 27 the following business was transacted:

Professor C. Y. Cannon, reporting on the Pasture Committee, requested that the committee be continued and that R. H. Lush, of the University of Louisiana, be reappointed as the association's representative on the joint committee with representatives of the Animal Production Society and the Agronomic Society.

Announcements of the appointment of Professor R. B. Stoltz as Secretary-Treasurer; of the reappointment of Dr. A. C. Dahlberg as Editor of the JOURNAL OF DAIRY SCIENCE; of the acceptance of the invitation to hold the 1936 meeting at Pennsylvania State College on June 17, 18 and 19; and of the adoption of the "Registration Fee" as a means of paying the expenses of holding the annual meeting.

The following report of the Production Section was given by Professor C. Y. Cannon, Chairman.

All papers were presented as listed. Average attendance per session 66.

Committee on Nominations were:

C. F. Huffman, Chairman, E. C. Elting and H. P. Davis. The committee presented the following names for election:

For Chairman—K. S. Morrow—(elected)		New Hampshire.
	R. H. Ruffner	North Carolina.
For Secretary—W. E. Kraus —(elected)		Ohio.
	S. I. Bechdel	Pennsylvania.

Committee on Resolutions were: Fordyce Ely, Chairman, R. B. Becker and T. M. Olson. The committee presented resolutions to the general Resolutions Committee.

Pasture report presented in mimeograph form.

Judging committee reported.

Report moved and accepted.

Judging Committee is as follows: I. W. Rupel, Wisconsin, E. C. Elting, South Carolina, Paul Reaves, Virginia, Elmer Hanson, Iowa, J. F. Kendrick, U. S. D. A., Washington, D. C.

Report of Committee on Breed Relations given by R. T. Harris of Wisconsin—moved, seconded and adopted that breeds take aliquot samples of each milking for composite testing.

Breeds Relation Committee appointed: Roy Harris, Chairman, Wisconsin, S. M. Salisbury, Ohio, Fordyce Ely, Kentucky, E. J. Perry, New Jersey, and C. N. Shepardson, Texas.

The Pasture Committee was reappointed as follows: I. R. Jones, Oregon, G. Bohstedt, Wisconsin, C. B. Bender, New Jersey, R. B. Becker, Florida, and R. H. Lush, Chairman.

The following report of the Extension Section was given by Floyd Johnson, Chairman:

The Extension Section held two half-day sessions and one short session for the explanation of exhibits of teaching materials.

The first session was held on Monday afternoon, June 24th, in order to complete the work of the section and give opportunity for extension members to hear as many papers as possible given by the other sections. The papers given were short reports of committees previously appointed to make studies of the best methods of developing and executing dairy extension projects.

The reports dealt with the following subjects: Exhibits, Quality Improvement in Dairy Products, Sires, Testing, Feeding and 4-H Clubs.

Time was set aside for discussion after each paper. Those attending took full advantage of this opportunity and at times this part of the program became lively.

The officers of the extension section were pleased to see so many members from the other sections attending our meetings.

About 50 attended the two half-day sessions and about 20 attended the short session for the explanation of exhibits.

Last year an exhibit of teaching materials and devices was made by the extension section. This project proved to be of such value that it was repeated this year. The exhibit this year was so much more complete and more interesting than that of last year that the section voted to continue this phase of the program.

There were exhibits from 15 States and the Bureau of Dairying at Washington. Exhibits of publicity materials and dark room was furnished so that film strips could be shown.

The extension section greatly appreciated the attitude of the general program committee in assisting with the making of the extension program.

This section also appreciated the excellent arrangements provided for exhibits and meetings.

The extension section passed a resolution commending the association for arranging for a section on problems and methods of instruction. The extension group feels that this idea should be further developed to include all phases of dairy teaching. We believe

that the teaching of agriculture and dairying in particular offers a real opportunity to provide education in its broadest sense.

The events of the last few years show that we need to educate leaders in dairying who are not only well trained in that field, but who are also broad-gauged members of society.

In 1934 there were 43 extension members of the American Dairy Science Association —in 1935 there were 56. Only 3 members failed to renew their membrship this year.

There are 110 dairy extension men in the United States according to extension lists furnished by the Bureau of Dairying.

Officers elected for 1936 were:

E. J. PERRY, New Jersey, *Chairman*
C. L. BLACKMAN, Ohio, *Vice-Chairman*
EARL SHULTZ, Iowa, *Secretary*

The following report of the Manufacturing Section was given by Professor G. N. Trout, Secretary:

REPORT OF THE DAIRY MANUFACTURERS' SECTION

Due to the able work of Chairman Mack and to the cooperation of the several members presenting papers, the full program of the Dairy Manufacturing Section has been carried out according to schedule. With the exception of two papers withdrawn, the program was carried out as printed.

At the business meeting, Dr. E. S. Guthrie, Chairman of the general committee on "Chemical Methods for the Analysis of Milk and Dairy Products," reported that progress was being made by each of the seven subcommittees on Milk, Butter, Cheese, Ice Cream, Condensed and Evaporated Milk, Dry Milk, and Skim Milk, Buttermilk, and Whey. He reported that four of the committees had been very active and would soon have their findings in published form.

Dr. Harold Macy, reporting for the general committee on "Bacteriological Methods for the Analysis of Milk and Dairy Products," stated that the committees on Milk, Butter, Cheese, Ice Cream, Condensed and Evaporated Milk, and Dry Milk had reported progress.

Prof. H. W. Gregory, chairman of the committee on "Judging Dairy Products," summaried the work of the committee and pointed out the interest being shown in the judging of dairy products as evidenced by the increase in the number of judging teams participating in the national contest.

Dr. H. E. Van Norman discussed briefly the educational work of the Chicago Mercantile Exchange. Chairman Mack appointed a committee, composed of Prof. M. Mortensen, chairman, Dr. O. F. Hunziker, and Prof. P. S. Lucas, to work with Dr. Van Norman to investigate the possibilities of a plan for recognizing the buttermaking efficiency of butter makers in the several states now sponsoring state scoring contests.

Dr. L. M. Thurston, West Virginia, and Dr. W. V. Price, Wisconsin, were elected chairman and secretary respectively for the Manufacturers Section for the coming year.

G. M. TROUT
Secretary, Manufacturing Section

A report of the hotel arrangements for the association in St. Louis at time of the National Dairy Show next October was given by Professor Ragsdale.

Professor Gregory gave a report on the Products Judging Contest.

There was some discussion concerning the possibilities of a banquet at the Dairy Show next fall at which both the dairy cattle contest winners and the products contest winners would be honored.

Mr. Van Norman offered the following motion—which was adopted—that Professors Gregory, Ruehe, and Ragsdale constitute a committee to work out arrangements for a joint banquet that would be in accordance with the sentiment expressed at this meeting.

Professor Fitch, Chairman of a Committee on Milk Standards, stated that the committee was not ready to report and requested that the committee be continued.

The following Report of the Resolutions Committee was read by Professor Borland and approved by the Association:

Whereas the American Dairy Science Association assembled at the University of Minnesota, College of Agriculture, University Farm, has enjoyed a most satisfactory convention professionally and socially, therefore be it resolved

(1) That the appreciation of the membership be tendered to the personnel of the University of Minnesota and College of Agriculture and to the following organizations which have contributed to the success of the 30th annual meeting:

Dairy Record.
Dairy Supply Houses.
General Mills, Incorporated.
Land O'Lakes Creameries, Incorporated.
Minneapolis Milk Distributors.
Minnesota Creamery Operators' and Managers' Association.
Northwest Ice Cream Association.
St. Paul Milk Distributors.
Twin City Milk Producers' Association.
Twin City Unit of the National Dairy Council.
Twin City Press.

(2) That in view of the fact that more attention is now being given to the fundamental breeding of all classes of live stock including dairy cattle, we recommend that more effort be directed toward the keeping of continuous production records of dairy cattle through the herd test and Dairy Herd Improvement Associations. We commend with favor any effort that can be made toward increasing financial support for this work from available State and Federal funds.

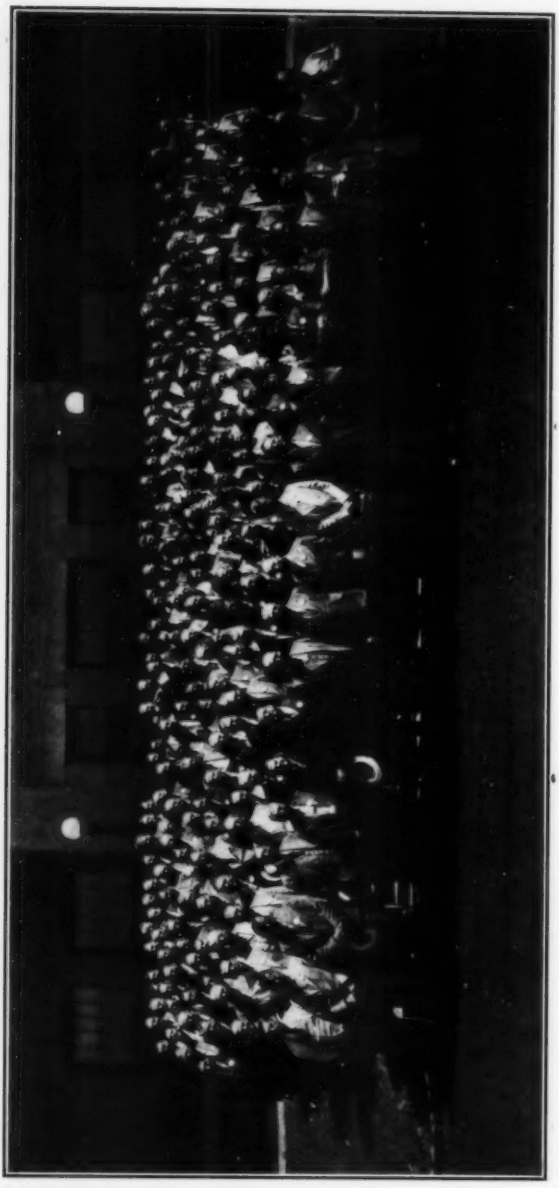
(3) That we earnestly urge that definite effort be made to improve the educational value of the dairy cattle judging contests as sponsored by this association and held in connection with the National Dairy Show. These contests in the past added much enthusiasm and interest among our students. We now have available knowledge which makes possible the development of a contest which will take into consideration the economic production ability and genetic make-up of our dairy cattle in addition to the appraisal of the value of dairy cattle on conformation. The form of contest approved by the American Dairy Science Association will influence the procedure followed in similar contests held under the direction of the 4-H club and vocational high school organizations.

(4) That we commend the Food and Drug Administration of the United States Department of Agriculture for its effort to improve the quality of dairy products and also commend the efforts of all organizations and individuals of the dairy industry that are making constructive efforts to aid in the improvement of the quality of all dairy products,

and further commend the payment to the producer of a differential for the delivery of quality milk and cream.

O. E. REED
H. E. VAN NORMAN
W. J. FRASER
J. A. NELSON
A. A. BORLAND, *Chairman*

Adjournment.



AMERICAN DAIRY SCIENCE ASSOCIATION MEMBERSHIP LIST AS OF JULY 15, 1935

A

ABBOTT, FRED H., Dairy Industry Division, College of Agri., Davis, Calif.
ACKERMAN, RICHARD A., Reymann Memorial Farms, Wardensville, W. Va.
ADAMS, HAROLD S., Nashoba Assoc. Board of Health, Town Hall, Ayer, Mass.
ADDINGTON, LAWRENCE H., New Mexico A. & M. College, State College, New Mexico.
AHLSTRAND, ERIC R., State Dairy & Food Inspector, Zumbrota, Minn.
AHLSTROM, WALTER, 3800 Lyncale Ave. N., Minneapolis, Minn.
AHRENS, ALFRED H., 1179 Forest Ave., Bronx, New York, N. Y.
ALEXANDER, C. B., The Akron Pure Milk Co., 273 Bowery St., Akron, Ohio.
ALGER, HARRY B., 13505 Griggs, Detroit, Mich.
ALLEN, ARTHUR E., 100 Shell St., Progress, Pa.
ALLEN, NAT. N., University Farm, St. Paul, Minn.
ALTWEGG, C. A., Lait Gloria Ste. Anne, 4 Rue Roussel, Paris 17e, France.
ANDERSON, D. L., Grays Harbor Dairy Products Co., Aberdeen, Wash.
ANDERSON, E. C., Conn. Agri. College, Storrs, Conn.
ANDERSON, E. E., New Mexico College of Agri., State College, New Mexico.
ANDERSON, GEORGE C., Cry. Pack. Mfg. Co., 1243 West Washington Blvd., Chicago, Ill.
ANDERSON, J. ARLINGTON, Dept. of Bact., Rutgers University, New Brunswick, N. J.
ANSBACHER, S., The Dry Milk Co., Inc., Bainbridge, N. Y.
ANSEL, ESTELL, R. F. D. No. 1, Circleville, O.
ANTHONY, E. L., Michigan State College, East Lansing, Mich.
AREY, J. A., State College Sta., Raleigh, N. C.
ARMSTRONG, TALBOTT V., 801 Cherry Ave. N. E., Canton, Ohio.
ARNOLD, P. T., Dept. of Animal Husb., Univ. of Florida, Gainesville, Fla.
ASTROTH, FRANK B., 1453 Hythe St., St. Paul, Minn.
ATKESON, F. W., Dairy Husb. Dept., Kansas State College, Manhattan, Kansas.
ATKINSON, L. A., Fraser Valley Milk Prod. Assn., 405 8th Ave., Vancouver, B. C., Canada.
AUSTIN, CARLTON J., 3756 Woodland Ave., Drexel Hill, Pa.
AYRES, HARVEY L., 7 University Road, College Park, Md.
AYRES, W. E., Dept. of Dairy Ind., Cornell Univ., Ithaca, N. Y.

B

BABCOCK, C. J., Bureau of Dairy Industry, U. S. D. A., Washington, D. C.
BAILEY, DANA H., 213 Dairy Bldg., State College, Pa.
BAISLER, WALTER, R. F. D. 2, Poland, Ohio.
BAKER, M. L., Sub. Station, North Platte, Nebr.
BAKER, M. P., Iowa State College Dairy Industry Dept., Ames, Iowa.
BALDERSTON, ROBT. W., National Dairy Council, 221 North LaSalle St., Chicago, Ill.
BALDWIN, FRANK BRUCE, Dairy Div., University Farm, St. Paul, Minn.
BALTZER, A. C., Extension Dairyman, Mich. State College, East Lansing, Mich.
BARBER, ALBERT H., c/o Cherry-Burrell Corp., 427 West Randolph St., Chicago, Ill.
BARBER, GEO. W., Pure Milk Co., Birmingham, Ala.
BARIL, W. A., Borden-Wieland Inc., 3638 Broadway, Chicago, Ill.
BARIM, MORRIS, Capitol Dairy, 3114 W. 16th St., Chicago, Ill.
BARKMAN, J. O., College of Agri., Lexington, Ky.
BARNES, JOHN C., c/o Twin City Milk Prod. Assn., 1001 Glenwood Ave., Minneapolis, Minn.

BARTLETT, J. W., Dairy Dept., N. J. Agri. College, New Brunswick, N. J.
BASS, LAWRENCE W., Borden Co., 350 Madison Ave., New York, N. Y.
BAUMANN, LEWIS A., c/o French Bauer, Cincinnati, Ohio.
BAYER, A. H., 710 Eastern Ave., Schenectady, N. Y.
BEAM, A. LELAND, Penn. State College, State College, Pa.
BECHDEL, S. I., Penn. State College, State College, Pa.
BECK, A. J., Walla Walla Dairymens Assn., Walla Walla, Wash.
BECKER, R. B., Animal Industry Dept., Univ. of Florida, Gainesville, Fla.
BELL, ARTHUR I., 206 Medical Arts Bldg., Baltimore, Md.
BELL, R. W., 4409 Greenwick Parkway, Washington, D. C.
BENDER, C. B., N. J. Agri. Expt. Sta., Dept. of Dairy Husb., New Brunswick, N. J.
BENDER, DR. RAYMOND, Box 212, Bainbridge, N. Y.
BENDIXEN, H. A., Dept. of Dairy Husb., State College of Wash., Pullman, Wash.
BENNETT, C. L., Frechtling Dairy Co., Front & Wilson St., Hamilton, Ohio.
BENNETT, F. W., Dairy Dept., Georgia State College of Agri., Athens, Ga.
BERRY, M. H., Dairy & Animal Husb. Dept., Univ. of Maryland, College Park, Md.
BESEMER, A. M., Golden State Milk Co. Ltd., 425 Battery St., San Francisco, Calif.
BIGELOW, A. P., 255 Homestead Ave., Hartford, Conn.
BIRD, EMERSON W., Dept. of Dairy Ind., Iowa State College, Ames, Iowa.
BIRD, JOHN WILLIAM, Box 25, Truro, N. S., Canada.
BLACKMAN, CHAS. L., Ohio State Univ., Columbus, Ohio.
BLAKE, E. SMYTH, Proprietor, Blake Dairy, Belton, S. C.
BODER, FRANCIS J., 432 River Road, Avalon, Pittsburgh, Pa.
BOEHR, JOHN W., Extension Division, Oklahoma A. & M. College, Stillwater, Okla.
BOHSTEDT, GUS, College of Agri., Agri. Hall, Madison, Wis.
BOISVERT, OSCAR, Ecole de Laiterie, St. Hyacinthe, P. Q., Canada.
BOONE, EUGENE, Route 2, Box 542, Modesto, Calif.
BOONE, IRWIN DEAN, 129½ Kern St., Box 492 Tulare, Calif.
BORLAND, A. A., Dairy Husb. Dept., Pennsylvania State College, State College, Pa.
BOULWARE, JAMES H., 405 Kipling St., Palo Alto, Calif.
BOUSKA, F. W., Beatrice Cry. Co., 1526 South State St., Chicago, Ill.
BOUSKA, FRANK J., Beatrice Cry. Co., Galesburg, Ill.
BOWLING, G. A., Dairy Dept., West Virginia Univ., Morgantown, W. Va.
BRANNON, J. M., Dairy Dept., Univ. of Illinois, Urbana, Ill.
BREED, ROBT. S., Agri. Expt. Sta., Geneva, N. Y.
BREMER, HARRY E., State House, Montpelier, Vt.
BREW, JAMES D., 201 Elmwood Ave., Ithaca, N. Y.
BRITT, JAMES A., City Consumers Co., Paducah, Ky.
BROCKSCHMIDT, J. H., Y. M. C. A., 13220 Woodward Ave., Highland Park, Mich.
BRODY, SAMUEL, Dairy Bldg., Univ. of Missouri, Columbia, Mo.
BROOKS, H. J., General Delivery, Suan Juan, Puerto Rico.
BROWN, ELIOT E., 227 University Ave., Davis, Calif.
BROWN, HAMLIN L., Extension Dairyman, Gainesville, Florida.
BROWN, J. C., American Assn. Cream Butter Mfgs., 1404 Chicago Mercantile Exch., Chicago, Ill.
BROWN, L. W., 2127 Commonwealth Ave., Madison, Wis.
BROWN, LEROY M., 5732 Pillsbury Ave. S., Minneapolis, Minn.
BROWN, ROYER H., c/o The Jersey Bulletin, 602 Majestic Bldg., Indianapolis, Ind.
BROWN, W. CARSON, College of Agri., Morgantown, W. Va.
BROWNELL, S. J., Animal Husb. Dept., New York State College of Agri., Ithaca, N. Y.
BRUECKNER, H. J., Dairy Dept., Cornell Univ., Ithaca, N. Y.

BULLOCK, KENNETH J., Southland Dairy Products Co., Inc., 60 Stockton St., Jacksonville, Fla.
BURGWALD, L. H., Dept. of Dairy Tech., Ohio State Univ., Columbus, Ohio.
BURKE, A. D., Alabama Polytechnic Inst., Auburn, Ala.
BURKHOLDER, J. K., 105 S. Hilton St., Decatur, Ill.
BURLINGAME, M. M., Borden's Farm Products Company of Mich., 3600 Forest Ave. E., Detroit, Mich.
BUTTON, F. C., New Jersey Agri. Expt. Sta., New Brunswick, N. J.
BUTTRICK, DAVID H., 30 Mill St., Arlington, Mass.

C

CAINE, GEO. B., Utah Agri. College, Logan, Utah.
CAMBURN, O. M., Vt. Agri. Expt. Sta., 489 Main St., Burlington, Vt.
CAMPBELL, M. H., Dept. of Animal & Dairy Husb., Univ. of Vermont, Burlington, Vt.
CANAN, R. D., 823 Lafayette Life Bldg., Lafayette, Ind.
CANNON, C. Y., Animal Husb. Dept., Iowa State College, Ames, Iowa.
CAPOUCH, FRANK J., c/o Bowman Dairy Co., 140–158 W. Ontario St., Chicago, Ill.
CARPENTER, DWIGHT C., New York State Agri. Expt. Sta., Geneva, N. Y.
CARR, GALE, c/o Shelby Co. Penal Farm, A. 6, Memphis, Tenn.
CARSON, ROBERT B., 606 11th Ave., Huntington, W. Va.
CARTER, E. B., Meadow-Gold Dairies, 304 S. Main St., Decatur, Ill.
CARTER, R. J., Moore-Ross Milk Co., Columbus, Ohio.
CARY, C. A., 215 Madison Ave., Riverdale, Md.
CASIDA, L. E., Dept. of Genetics, College of Agri., Madison, Wis.
CATHCART, S. L., Iberia Livestock Expt. Sta., Jeanerette, La.
CAUFIELD, W. J., Dept. of Dairy Husb., College of Agri., Manhattan, Kansas.
CAVE, H. W., Dairy Dept., College of Agri., Manhattan, Kansas.
CHAMBERS, LESLIE A., Johnson Foundation, University of Pennsylvania, Philadelphia, Pa.
CHAPMAN, LEROY M., 293 White St., Danbury, Conn.
CHARLES, DONALD I., Ohio State Univ., Columbus, Ohio.
CHERBONNIER, E. G., Purina Mills, 8th and Gratoit Sts., St. Louis, Mo.
CHRISTENSEN, SOREN, 1131 10th St., Modesto, Calif.
CLARK, JOHN I., Blairsville Sanitary Dairy Co., 201 Wynn St., Blairsville, Pa.
CLEVENGER, C. L., Mount Hope Farm, Williamstown, Mass.
CLEVENGER, W. L., Dairy Dept., State College, Raleigh, N. C.
COHEE, C. I., 219 N. Broad St., Philadelphia, Pa.
COLE, CLARENCE L., North Central School and Station, Grand Rapids, Mich.
COLE, WILFORD C., College of Agri., Davis, Calif.
COLLINS, HOMER A., Mutual Cry. Co., Salt Lake City, Utah.
COLLINS, DR. MERVYA, Dairy Corp., 80 King St. N. W., Toronto, Canada.
COLMAN, H. N., Dairy Dept., College of Agri., Corvallis, Ore.
COLTERYAHN, CARL A., 1614 Brownsville Road, Pittsburgh, Pa.
COMBS, W. B., Dairy Husb. Div., University Farm, St. Paul, Minn.
CONKLIN, C. T., Sec., Ayrshire Breeders Assn., Brandon, Vt.
CONNELLY, R. G., Dept. of Dairying, College of Agri., Blacksburg, Va.
COOK, A. S., Walker Gordon Laboratory, Plainsboro, N. J.
COOK, L. RUSSELL, 19 W. Lemon St., Lititz, Pa.
COOLEY, LOUIS A., Mgr., Country Dept. Whiting Milk Co., 570 Rutherford Ave., Boston, Mass.
COPELAND, LYNN, American Jersey Cattle Club, 324 W. 23rd St., New York, N. Y.
CORBETT, L. S., Orono, Maine.

CORBIN, C. I., 524 W. 57th St., New York, N. Y.
CORDES, WILLIAM A., Blue Valley Cry. Co., 1137 S. Jackson Blvd., Chicago, Ill.
COULTER, S. T., University Farm, St. Paul, Minn.
COWAN, ROBERT, 635 Elliott Ave. West, Seattle, Washington.
COX, C. G., 44102½ Drexel Blvd., Chicago, Ill.
CRAMER, A. J., 522 N. Pickney St., Madison, Wis.
CRANDALL, A. T., Jonesville, Vt.
CRANDALL, W. T., New York State College of Agri., Cornell Univ., Ithaca, N. Y.
CREAMER, DWIGHT S., 2139 Abington Road, Cleveland, Ohio.
CRONE, H. E., 229 Bourbon Stock Yards, Falls Cities Coop. Milk Producers Assn., Louisville, Ky.
CROSBY, J. E., Jr., Dairy Dept., Univ. of Missouri, Columbia, Mo.
CROWE, L. K., Dairy Dept., Univ. of Nebraska, Lincoln, Nebr.
CUMMINGS, C. M., The American Guernsey Cattle Club, Peterboro, N. H.
CUNNINGHAM, W. S., University of Ariz., Tucson, Ariz.
CUNNINGHAM, G. C., Dairy Dept., College of Agri., State College of New Mexico, State College, New Mexico.

D

DAHLBERG, A. C., Agri. Experiment Sta., Geneva, N. Y.
DAHLE, CHESTER D., Dept. of Dairy Husb., Penn. State College, State College, Pa.
DARNELL, A. L., College Station, Texas.
DAVIS, GLENN G., Fredmar Farms, Jefferson Barracks, Mo.
DAVIS, H. P., Dairy Dept., College of Agri., Lincoln, Nebr.
DAVIS, HOWARD D., 2804 Huey Ave., Drexel Hill, Pa.
DAVIS, MYRON C., Alpha Gamma Rho Frat., 90 Pleasant St., Amherst, Mass.
DAVIS, R. N., College of Agri., Tucson, Ariz.
DELISLE, RODNEY, 2195 West 40th St., Vancouver, B. C., Canada.
DEMETER, KARL J., Post Freising, Obb, Weihenstephan, Germany.
DEY, MARCUS, Golden State Co., Ltd., 1120 Towne Ave., Los Angeles, Calif.
DICE, J. R., State College Station, Fargo, N. D.
DICKERSON, GORDON E., Farm and Dairy Records Office, 207 King Hall, Madison, Wis.
DICKEY, HOWARD, Dairy Dept., W. Va. Univ., Morgantown, W. Va.
DICKSON, R. W., Virginia Polytechnic Inst., Blacksburg, Va.
DOAN, F. J., Dairy Dept., Pennsylvania State College, State College, Pa.
DORCAS, M. J., 362 Front St., Berea, Ohio.
DORMAN, D. M., 325 Roosevelt Bldg., Los Angeles, Calif.
DORSEY, L. M., Dairy Division, Univ. of Maine, Arono, Me.
DOTTERRER, W. D., c/o Bowman Dairy Co., 140–158 W. Ontario St., Chicago, Ill.
DOWNS, P. A., Dept. of Dairy Husb., Univ. of Nebraska, Lincoln, Nebr.
DREYER, VIRGIL O., 219 East Fifth St., Dayton, Ohio.
DWYER, GEORGE L., Box 386, Waterbury, Vt.

E

EASTERN STATES FARMERS' EXCH., P. O. Box 1482, Springfield, Mass.
EASTWOOD, H. S., DeLaval Separator Co., 165 Broadway, New York, N. Y.
EDWARDS, FRANCIS REES, Rept. Animal Industry, Georgia Expt. Sta., Experiment, Ga.
ELDRED, R. E., Dairy Div., Univ. Farm, St. Paul, Minn.
ELLENBERGER, H. B., University of Vermont, Burlington, Vt.
ELLINGTON, E. V., Daity Dept., Washington State College, Pullman, Wash.
ELTING, E. C., Clemson A. & M. College, Clemson College, S. C.
ELY, FORDYCE, University of Kentucky, Lexington, Ky.

ENGLAND, C. W., Dairy Bldg., Univ. of Maryland, College Park, Md.
ERB, J. HOFFMAN, College of Agri., Ohio State Univ., Columbus, Ohio.
ERF, OSCAR, Ohio State Univ., Townsend Hall, Columbus, Ohio.
ESPE, DWIGHT L., 612 Lynn Ave., Ames, Iowa.
ESPE, O. W., Broadview Dairy Co., Spokane, Wash.
EVERETT, ROBERT, Dairy & Ice Cream Mch. & Supply Assn., Inc., 232 Madison Ave., New York, N. Y.

F

FABIAN, F. W., Div. of Vet. Science, Mich. Agri. College, East Lansing, Mich.
FALCONER, DAVID B., Scottsville, Mich.
FARRALL, A. W., c/o Cry. Pack. Mfg. Co., 1243 W. Washington St., Chicago, Ill.
FAWCETT, CLIFFORD, Mass. State College, Amherst, Mass.
FAY, A. C., Kansas State College, Manhattan, Kansas.
FENTON, F. E., Inland Empire Dairy Assn., Spokane, Wash.
FERRARI, CHARLES G., 4721 18th Ave., S. Minneapolis, Minn.
FIELDER, H. S., Cherry Burrell Corp., 427 W. Randolph St., Chicago, Ill.
FISHER, R. C., R. F. Worden & Co., Waterbury, Conn.
FITCH, J. B., University Farm, St. Paul, Minn.
FITTS, F. B., Dept. of Agri. Extension, 213 Dairy Bldg., Pa. State College, State College, Pa.
FLACK, M. L., Dairy Husb. Dept., College of Agri., Lincoln, Nebr.
FLANIGAN, GEORGE E., Box 168, Bainbridge, N. Y.
FLANLEY, MABEL G., 4419 White-Henry Stuart Bldg., Seattle, Wash.
FLEMING, R. S., The Borden Co., Syracuse, N. Y.
FLESHMAN, C. L., Va. A. & M. College, Blacksburg, Va.
FOLEY, DAN, 617 Main St., Vallejo, Calif.
FOLEY, RICHARD C., Farm House, Mass. State College, Amherst, Mass.
FOSKETT, CLIFFORD R., 50 Laurel St., East Weymouth, Mass.
FOURT, D. L., Univ. Extension Division, College of Agri., Boise, Idaho.
FRANDSEN, J. H., Mass. State College, Amherst, Mass.
FRARRAR, ROBERT R., Bureau of Dairy Industry, U. S. D. A., Washington, D. C.
FRASER, W. J., Univ. of Illinois, Urbana, Ill.
FRAYER, JAMES M., Agri. Expt. Sta., 489 Main St., Burlington, Vt.
FRAZIER, WILLIAM C., 742 Miami Pass, Madison, Wis.
FREDERIKSEN, FELIX M., 725 McIndoe St., Wausau, Wis.
FRENCH, H. A., P. O. Box 862, Uniontown, Pa.
FRETWELL, C. B., Drawer 972, Spartanburg, S. C.
FREVERT, G. E., Del Norte Milk Products Co., Smith River, Calif.
FROHING, W. O., 4614 Prospect Ave., Cleveland, Ohio.
FRY, BERNICE L., College of Agri., Davis, Calif.
FRYHOFER, C. W., Land O'Lakes Creameries, Inc., 2201 Kennedy St. N. E., Minneapolis, Minn.
FULLER, STEWART A., College of Agri., Davis, Calif.

G

GAINES, W. L., Dept. of Dairy Husb., Univ. of Illinois, Urbana, Ill.
GALEMA, M. L., 2504 183rd St., Oak Glen, Ill.
GANNON, E. A., Purdue Univ., Lafayette, Ind.
GARDNER, W. U., Dept. of Anatomy, Yale Univ., New Haven, Conn.
GARRISON, E. R., Dairy Dept., College of Agri., Columbia, Mo.
GAUNTT, E. A., Extension Dairyman, New Jersey Agri. College, New Brunswick, N. J.

GAVIN, JOSEPH S., 200 Stockbridge Ave., Buffalo, N. Y.
GEARHART, C. R., State College, State College, Pa.
GEBHARDT, H. T., 418 Milford Ave., Marysville, Ohio.
GELBMAN, MILTON, 2209 Oak St., Jacksonville, Fla.
GENTRY, J. BRYAN, Box 83, Hobart, Okla.
GEORGIDSE, AMBERSY, Lab. & Mfg. Dept., Grandview Dairy Inc., Webster's Crossing, Livingston, N. Y.
GIFFORD, WARREN, 233 Marion Ave., Clarendon, Va.
GOLDING, N. S., College of Agri., Pullman, Wash.
GOODALE, B. E., Clemson College, S. C.
GOOLD, IVA A., 218 Center St., East Lansing, Mich.
GORDON, E. F., Dept. of Dairy Husb., Iowa State College, Ames, Iowa.
GORDON, G. E., College of Agri., Davis, Calif.
GOSS, E. F., Dept. of Dairy Husb., Iowa State College, Ames, Iowa.
GOSS, W. G., 727 Seward St., Evanston, Ill.
GRAVES, R. R., Bureau of Dairy Industry, U. S. D. A., Washington, D. C.
GRAY, C. E., 6263 Chabot Road, Oakland, Calif.
GREENBACKER, HERBERT A., Murdock Ave., Meriden, Conn.
GREENE, SAM H., c/o Calif. Dairy Council, 216 Pine St., San Francisco Calif.
GREER, D. M., Lawson Y. M. C. A., 30 West Chicago Ave., Chicago, Ill.
GREGORY, H. W., Chief of Dairy Husb., Purdue Univ., Agri. Expt. Sta., Lafayette, Ind.
GRIESS, WALTER RICHARD, 19 West 7th St., Cincinnati, Ohio.
GRIMES, MICHAEL, Dept. of Dairy Bact., Univ. College, Cork, Irish Free St.
GROW, WALTER, Mgr. Fluid Milk Plant, 265 Beech St., Pottstown, Pa.
GULLICKSON, T. W., Univ. of Minn., St. Paul, Minn.
GUTHRIE, E. S., Dept. of Dairy Industry, Cornell University, Ithaca, N. Y.

H

HADLEY, I. D., 152 N. Columbus St., Wooster, Ohio.
HAIGHT, R. A., Swift & Co., Produce Dept., South St. Paul, Minn.
HAINES, GEORGE, Office of Expt. Stations, Washington, D. C.
HALE, JOSEPH C., R. D. No. 1, Silver Lake, Cuyahoga Falls, Ohio.
HALES, M. W., Chr. Hansen's Labs. Inc., 9015 West Maple St., Milwaukee, Wis.
HALESKI, FRANK, 30 West 85th St., New York, N. Y.
HALL, WARREN C., Dept. of Dairy Husb., Univ. of Missouri, Columbia, Mo.
HAMILTON, ROBERT, Cleveland Ice Cream Co., 47th 'Train, Cleveland, Ohio.
HAMMER, B. W., Iowa State College, Ames, Iowa.
HANAWALT, E. M., P. O. Box 540, Massillon, Ohio.
HANKINSON, CLOYCE L., Dairy Dept., Univ. of Calif., Davis, Calif.
HANSEN, A. P., Statens Forsogsmejeri, Hillerod, Denmark.
HANSON, CLEMENTS C., Mgr. Barnum Cry. Co., Barnum, Minn.
HANSON, E. A., University Farm, St. Paul, Minn.
HANSON, E. O., Vander Bie's Inc., St. Paul, Minn.
HANSON, F. E., Madison, Wis. (College of Agri., Univ. of Wisconsin).
HARDELL, ROBERT, 1517 15th Ave., Monroe, Wis.
HARDING, H. A., Box 517, Detroit, Mich.
HARDING, H. G., 273 Bowery St., Akron, Ohio.
HARDING, J. M., Harding Cream Co., Omaha, Neb.
HARFORD, ZAIDA M., The Dry Milk Research Lab., Box 535, Bainbridge, N. Y.
HARMON, E. M., 7112 7th St. N. W., Washington, D. C.
HARRIMAN, L. A., Dairy Industry Dept., Iowa State College, Ames, Iowa.

HARRIS, J. L., Box 173, Kouts, Ind.
HARRIS, GEORGE M., Dairy Bldg., 730 Rose St., Lexington, Ky.
HARRIS, ROY T., Dairy Records Office, Madison, Wis.
HARRISON, F. B., Dept. of Animal Husb., Cornell Univ., Ithaca, N. Y.
HARRISON, HARTMAN K., Linden Ave. & Dolphin St., Baltimore, Md.
HARTZ, B. P., 5509 Edgemoor Lane, Bethesda, Md.
HASTINGS, E. G., Univ. of Wisconsin, Agri. Hall, Madison, Wis.
HAYDEN, C. C., Expt. Sta. Wooster, Ohio.
HAYS, G. J., 213 Bailey St., East Lansing, Mich.
HEADLEY, F. B., Agri. Expt. Sta., Univ. of Nevada, Reno, Nevada.
HEADLEY, FREDERICK B., College of Agri., Univ. of Wisconsin, Madison, Wis.
HEALY, HENRY A., Dairy Products Labs., 2267 Como Ave., West, St. Paul, Minn.
HEDRICK, THEODORE, Bozeman, Mont.
HEFFERMAN, H. N., Reg. Sec., The Certified Milk Producers Assn., of America, 9269 Colbert St., New Orleans, La.
HEFFERMAN, HENRY JR., Dept. of Agri. Dairy & Food, State Office Bldg., St. Paul, Minn.
HEINEMAN, HENRY E. O., Milk Plant Monthly, 327 S. LaSalle St., Chicago, Ill.
HELMAN, J. L., 810 Barnett St., Syracuse, N. Y.
HENDERSON, H. O., College of Agri., Morgantown, W. Va.
HENDERSON, J. FLOYD, College Park, Davis, Calif.
HENING, J. C., New York Expt. Sta., Geneva, N. Y.
HERMAN, HARRY, A., Dept. of Dairy Husb., College of Agri., Columbia, Mo.
HERREID, ERNEST, Div. of Dairy Husb., Univ. Farm, St. Paul, Minn.
HERRICK, RICHARD G., 4120 Sheridan Ave. S., Minneapolis, Minn.
HERRINGTON, B. L., Dept. of Dairy Industry, Cornell Univ., Ithaca, N. Y.
HERRINGTON, FRED L., 1837 Fell St., Enumclaw, Wash.
HERRMAN, LOUIS F., 400 Stewart St., Morgantown, W. Va.
HIBBEN, R. C., International Association of Ice Cream Mfgrs., 1105 Barr Bldg., Washington, D. C.
HIBBERT, C. W., Challenge Cream & Butter Assn., 925–929 East Second St., Los Angeles, Calif.
HIDINGER, ORVIS, 205 Times Bldg., Scranton, Pa.
HIGGINS, JAMES L., Summit Cry., Oconomowoc, Wis.
HIGGINS, L. A., Starkville, Miss.
HILEMAN, J. L., 810 Burnet Ave., Syracuse, N. Y.
HILL, OTTO J., Extension Service, College of Agri., Pullman, Wash.
HILTON, J. H., Dept of Dairy Husb., Purdue Univ., Lafayette, Ind.
HINMAN, ELMER S., North Walnut St., Spring Grove, Pa.
HIXON, R. F., 608 N. Babcock St., Bozeman, Mont.
HODGSON, R. E., Western Washington Expt. Sta., Puyallup, Wash.
HOFFMAN, JOHN, c/o Nestles Milk Prod., Inc., Burlington, Wisc.
HOLDAWAY, C. W., Dept. of Dairy Husb., Virginia Polytechnic Inst., Blacksburg, Va.
HOLDER, EVEN D., 27 Florence St., Hudson, Mass.
HOLZHAUER, HENRY K., Moss Dairy Farm Inc., Chesterland, Ohio.
HOMBERGER, ROBERT E., 1418 Hayes Ave., Sandusky, Ohio.
HOOD, E. G., Dairy Commissioner's Office, Ottawa, Canada.
HOOPER, H. A., Dept. of Animal Industry, Cornell Univ., Ithaca, N. Y.
HORROLL, B. E., Purdue Univ., Dairy Husb. Dept., Lafayette, Ind.
HORWOOD, RUSSEL E., Expt. Station, Chatham, Mich.
HOWARD, RALPH, c/o American Stores Co., 1028 New York Bldg., St. Paul, Minn.

HOWAT, R. J., The Dry Milk Industry, 221 North LaSalle St., Chicago, Ill.
HOWE, D. K., Fairmont Cry. Co., Omaha, Nebr.
HOWE, JOHN, Hamilton, Mont.
HUCKER, G. J., N. Y., State Agri. Expt. Sta., Geneva, N. Y.
HUFFMAN, CARL F., Dairy Dept., Mich. Agri. College, East Lansing, Mich.
HUNZIKER, O. F., Blue Valley Cry. Co., Chicago, Ill.
HURST, ROBERT N., 1602 Neil Ave., Columbus, Ohio.
HUSSONG, R. V., Dairy Ind. Dept., Iowa State College, Ames, Iowa.

I

IDDINGS, HARRY G., 2937 North 59th St., Omaha, Nebr.
ISLAY, S. D., 1033 Mahoning Ave., Youngstown, Ohio.
ITKIN, M. G., c/o Agrojoint, Moskau, Staropimenowsky per 14, Russia.
IVERSON, C. A., Dept. of Dairying, Iowa State College, Ames, Iowa.

J

JACKSON, GEORGE F., 5414½ S. Denker Ave., Los Angeles, California.
JACKSON, H. C., Dairy Dept., Univ. of Wisconsin, Madison, Wis.
JACOBSEN, D. H., Dept. of Dairy Ind., Iowa State College, Ames, Iowa.
JANZEN, HAROLD W., Marigold Dairies, Red Wing, Minn.
JENSEN, CHRIS, Dairy Dept., State College Sta., Fargo, N. Dak.
JERTSON, E. C., Krem-Ko Co., 4830 S. Turner Ave., Chicago, Ill.
JEWELL, R. M., 618 East High St., Mt. Vernon, Ohio.
JOHE, WM., 8556 Cashio St., Los Angeles, Calif.
JOHNS, C. K., Asst. Agri. Specialist, Central Expt. Farm, Ottawa, Canada.
JOHNSON, ALEXANDER, 2920 Clinton Ave. S., Minneapolis, Minn.
JOHNSON, R. E., Conn. Agri. College, Storrs, Conn.
JOHNSTON, FLOYD, College of Agri., Ames, Iowa.
JONES, FRANK L. SEYMOUR, c/o The Borden Company, 350 Madison Ave., New York, N. Y.
JONES, IDWAL R., Dairy Dept., Oregon Agri. College, Corvallis, Ore.
JONES, W. F., Ottawa Dairy Ltd., Ottawa, Canada.
JORDAN, H. C., Carlisle, Ark.
JUDD, WALLACE M., R. 1, Kinsman, Ohio.
JUDKINS, H. F., Nat. Dairy Products, 120 Broadway, New York, N. Y.

K

KEAY, JOHN, 10717 100 Ave., Edmonton, Alberta, Canada.
KEENEY, MARK H., Essex County Hospital, Cedar Grove, N. J.
KEILLING, JEAN, Regional Station of Dairy Research, Poligny (Jura), France.
KEITH, J. I., Dairy Husb. Dept., Oklahoma A. & M. College, Stillwater, Okla.
KEITHLEY, J. R., 2201 Scudder Ave., St. Paul, Minn.
KEITHLEY, R. P., Virginia Polytechnic Inst., Blacksburg, Va.
KELLY, C. D., Old Tavern, 2–20 Danforth St., Portland, Maine.
KELLY, ERNEST, Bureau of Dairy Industry, U. S. D. A., Washington, D. C.
KELLY, FRANK W., c/o Gridley Dairy Co., 620 N. 8th St., Milwaukee, Wis.
KELLY, THOMAS, 110 Llandaff Road, Llanerch, Pa.
KENDRICK, J. F., Bureau of Dairy Industry, U. S. D. A., Washington, D. C.
KIMBERLIN, OLIN E., 935 19th St., Merced, Calif.
KING, CHARLES GLEN, Chemistry Dept., University of Pittsburgh, Pittsburgh, Pa.
KNAYSI, GEORGES, Dairy Bldg., Cornell Univ., Ithaca, N. Y.

KNOOP, C. E., The Ohio Agri. Expt. Sta., Wooster, Ohio.
KNOTT, J. C., Dept. of Dairying, State College of Agri., Pullman, Wash.
KOCHER, DANIEL S., Ames Farm Dairy Co., 31st and Gillham Road, Kansas City, Mo.
KOEMAN, E. J., Gen. Mgr., Marigold Dairies Inc., 406 N. Broadway Ave., Rochester, Minn.
KRAUSS, WILLIAM E., Ohio Agri. Expt. Sta., Wooster, Ohio.
KREHBIEL, LEONARD F., 3720 Hughes Ave., Palmo, Calif.
KRUMPAH, MELVIN, c/o Issaquah Cry. Co., Issaquah, Wash.
KUHLMAN, A. H., Dairy Dept., Oklahoma A. & M. College, Stillwater, Okla.
KYLE, WILLIAM A., Ayrshire Breeders Association, Brandon, Vt.
KYZIVAT, JOHN L., 1002 N. Mariposa Ave., Los Angeles, Calif.

L

LACOSTA, CARLOS, Dorado, Porto Rico.
LAMASTER, J. P., Div. of Dairying, Clemson College, S. C.
LAMB, LELAND W., Dairy Husb. Dept., Mich. State College, East Lansing, Mich.
LAUTERBACK, A. H., Inter-State Milk Producers, 219 N. Broad St., Philadelphia, Pa.
LARSEN, C., State College, Brookings, S. D.
LARSEN, HUGO J., 3301 Highland Ave., Drexel Hill, Pa.
LARSON, C. W., 235 Elm St., Buffalo, N. Y.
LATZER, R. L., 1401 Arcade Bldg., St. Louis, Mo.
LEHMKUHL, HENRY, 73 Howell St., Rochester, N. Y.
LEIGHTON, ALAN, P. O. Box 5, Brentwood, Md.
LEIGHTON, RAMER, Univ. Farm, St. Paul, Minn.
LEITCH, RENWICK, West of Scotland Agri. College, Dairy Research Labs., Auchenerieve, Ayrshire, Scotland.
LESCURE, J. M., Division of Farm Insp., Room 900, Sanitary Section, Municipal Office, Baltimore, Md.
LICHTENBERGER, B., 71 Onderdonk Ave., Manhasset, L. I., N. Y.
LIDE, B. M., 2008 Pine St., St. Louis, Mo.
LINDQUIST, HARRY G., Dept. of Dairy Industry, Mass. State College, Amherst, Mass.
LINN, J. W., Extension Div., College of Agri., Manhattan, Kansas.
LIST, W. H., Association of Ice Cream Mfgs., Hotel Pennsylvania, 7th & 34th St., New York, N. Y.
LITTLE, LAWRENCE, Great Bend, Kansas.
LITTLE, R. E., Sec. International Milk Dealers Assn., 309 W. Jackson Blvd., Chicago, Ill.
LIVERS, HERBERT B., 323 Second Ave., Escondido, Calif.
LOCHRY, H. R., P. O. Box 251, Sugar Creek, Ohio.
LOUGHARY, IVAN H., Dairy Husb. Dept., University of Idaho, Moscow, Idaho.
LOVELAND, E. H., 101 Henry St., Burlington, Vt.
LUCAS, P. S., Dairy Dept., College of Agri., East Lansing, Mich.
LUSH, JAY L., Agricultural Hall, Iowa State College, Ames, Iowa.
LUSH, R. H., Agri. Expt. Sta., La. State University, Baton Rouge, La.
LYONS, THEODORE A., 78 Beechmont Ave., Bridgeport, Conn.

M

MACK, M. J., Mass. State College, Amherst, Mass.
MACY, HAROLD, Dairy Husb. Dept., St. Paul, Minn.
MALLOY, LOUIS J., Gr. Malloy's Dairy, 326 West Centre St., Shenandoah, Pa.
MANN, ALBERT I., Dairy Dept., Conn. Agri. College, Storrs, Conn.
MANUS, LOUIS J., Dept. of Dairy Husb., College of Agri., Morgantown, W. Va.

MANY, RUSSEL M., Borden Farm Products Co. of Michigan, 14707 Dexter Blvd., Detroit, Mich.
MARCUSSEN, W. H., Borden's Farm Prod. Co. Inc., 110 Hudston St., New York, N. Y.
MARTIN, WILLARD H., Dairy Husb. Dept., Kansas Agri. College, Manhattan, Kan.
MATHIESEN, H. A., 2033 N. E. Tillamook, Portland, Ore.
MATTHEWS, JOHN S., Intermediate Reformatory, Dept. of Penal Inst., Jefferson City, Mo.
MATTSON, H. A., Prod. Mgr. Ives Ice Cream Co., 128 Univ. Ave. S. E., Minneapolis, Minn.
MAYNARD, L. A., Laboratory of Animal Nutrition, Cornell Univ., Ithaca, N. Y.
MCBRIDE, C. S., Detroit Cry. Co., 306 E. Scott St., Grand Ledge, Mich.
MCBRIDE, CHARLES GROVER, 1644 Guilford Road, Columbus, Ohio.
MCCAN, J. C., New Jersey Agri. Expt. Sta., New Brunswick, N. J.
MCCHESNEY, E. R., Fairmont Cry. Co., Buffalo, N. Y.
MCCLEAN, J. A., 141 W. Jackson Blvd., Chicago, Ill.
MCCLELLAND, W. T., Shamrock Dairy, Tucson, Ariz.
MCCOWN, C. Y., Research Dept., Creamery Package Mfg. Co., 1243 E. Washington Blvd., Chicago, Ill.
MCCOWN, E. R., Fairmont Cry. Co., Buffalo, N. Y.
MCCUBBIN, W. G., Dairy Husb. Dept., Texas A. & M. College Station, Texas.
MCDONALD, CHARLES, 66 E. Northwood Ave., Columbus, Ohio.
MCDOWELL, J. C., Bureau of Dairy Industry, U. S. D. A., Washington, D. C.
MCGILLARD, P. C., Dairy Dept., Oklahoma A. & M. College, Stillwater, Okla.
MCINTYRE, C. W., Hatch Farm, Oakwood, Mo.
MCKINNEY, WM. B., 44 Lester Place, New Rochelle, N. Y.
MEAD, SYLVESTER, W., c/o Agri. Expt. Sta., Davis, Calif.
MEIGS, E. B., 1736 M. St. N. W., Washington, D. C.
MEISTER, ALBERT, 323 16th St., Sacramento, Calif.
MELGAARD, ARTHUR, 2102 Carter Ave., St. Paul, Minn.
MERRILL, A. R., Extension Dairyman, Conn. Agri. College, Storrs, Conn.
MERRILL, R. S., c/o Sego Milk Products Co., Richmond, Utah.
MILLER, DALE, Plant Supt. Circle City Dairy, Circleville, Ohio.
MILLER, NORMAN J., Mead Johnson & Co., Evansville, Ind.
MILLER, PAUL G., Dairy Division, University Farm, St. Paul, Minn.
MILNER, F. W., Room 204 State Bldg., Los Angeles, Calif.
MILONE, NICHOLAS A., Bureau of Milk Sanitation, State Dept. of Health, Albany, N. Y.
MINKIN, JOSEPH, 482 Donaldson St., Columbus, Ohio.
MIYAWAKI, A., Hokkaido Imperial Univ., Sapporo, Japan.
MOLDAVAN, ANDREW, 1025 Aqueduct St., Montreal, Quebec.
MONIER, HOWARD B., Box A College Station, Berea, Ky.
MONRAD, K. J., Chr. Hansen's Lab. Inc., 9015 W. Maple St., Little Falls, N. Y.
MONROE, C. F., Ohio Agri. Expt. Sta., Wooster, Ohio.
MOORE, HERBERT C., Univ. of New Hampshire, Durham, N. H.
MOORE, J. S., Agricultural College, Miss.
MOORE, L. A., Dairy Dept., Mich. State College, East Lansing, Mich.
MOORE, L. G., The Moore Dairy, Box 1074, Lancaster, Pa.
MORGAN, NORMAN C., 501 Viston St., Pomona, Calif.
MORLEY, LEWIS W., American Jersey Cattle Club, 324 W. Twenty Third St., New York, N. Y.
MORRIS, A. J., Dept. of Dairy Husb., Utah State College, Logan, Utah.
MORRISON, F. B., Dept. of Animal Husb., Cornell Univ., Ithaca, N. Y.

MORRISON, HENRY, Dairy Dept. Univ. of Kentucky, Lexington, Ky.
MORROW, KENNETH S., Dairy Husb. Bldg., Univ. of New Hampshire, Durham, N. H.
MORSE, ROGER W., College of Agri. Corvallis, Ore.
MORTENSEN, FRANCIS N., Richfield, Utah.
MORTENSEN, M., Iowa State College, Ames, Iowa.
MOSELEY, THOMAS W., Beltsville Expt. Farm, Beltsville, Md.
MOSLEY, W. K., 3730 E. New York St., Indianapolis, Ind.
MUDGE, COURTLAND S., University Farm Davis, Calif.
MUELLER, W. S., Dept. of Dairy Industry, Mass. State College, Amherst, Mass.
MURPHY, O. C., 2300 McCoy St., Kansas City, Mo.
MYERS, R. P., 1403 Eutaw Place, Baltimore, Md.

N

NAFIS, LOUIS F., 111 North Canal St., Chicago, Ill.
NAGOETTE, JOE, Dairy Extension Specialist, Dairy Bldg., State College, Pa.
NEAL, WAYNE M., Dept. of Animal Husb., Agri. Expt. Sta., Gainesville, Fla.
NELSON, D. H., Dairy Division, University Farm, Davis, Calif.
NELSON, J. A., Dept. of Dairy Industry, College of Agri., Bozeman, Mont.
NESBIT, J. C., Hoard's Dairyman, Ft. Atkinson, Wis.
NEVINS, W. B., College of Agr., Urbana, Ill.
NEWLANDER, J. A., University of Vermont, Burlington, Vt.
NEWMAN, WALTER A., 407 Sixth St. West, Huntington, W. Va.
NICOLET, BEN H., Bureau of Dairy Industry, Beltsville, Md.
NORRIS, L. C., Dept. of Poultry Husb., Cornell Univ., Ithaca, N. Y.
NYSTROM, A. B., Bureau of Dairy Industry, U. S. D. A., Washington D. C.

O

OLSON, HAROLD C., Dairy Industry Dept., College of Agri., Ames, Iowa.
OLSON, T. M., College of Agri., Brookings, S. Dak.
OSBORNE, R. F., 1450 Allison Ave., Los Angeles, Calif.
OTTING, H. E., R. D. 3, Westerville, Ohio.
OTTO, B. FRANK, Otto Suburban Dairy Inc., Camp Horne Road, Box 4198, Bellevue, Pitssburgh, Pa.

P

PALEY, CHAS., c/o Certified Labs. Inc., 19 Hudson St., New York, N. Y.
PALMER, LEROY S., Dept. of Dairy Husb., University Farm St. Paul, Minn.
PARAFITT, E. H., Purdue Univ. Dairy Dept., West Lafayette, Ind.
PARKER, HORATIO N., Engineering Bldg., Main & Orange Sts., Jacksonville, Fla.
PARKER, MILTON E., 123 N. Washington Ave., Danville, Ill.
PARKIN, IVAN, Room 2113, Dairy Extension, Penn State College, State College, Pa.
PARKS, J. R., 5413 Hartford Road, Baltimore, Md.
PEARCE, SIDNEY, Weber Central Dairy Assn., Ogden, Utah.
PERKINS, A. E., Experiment Station, Wooster, Ohio.
PERRY, E. J., New Jersey Agri. College, New Brunswick, N. J.
PESCHKEN, CLAIRE EDWARD, 1422 Knox Ave. N., Minneapolis, Minn.
PETERS, GEORGE T., Grassy Valley Farm & Dairy, Rt. 7, Knoxville, Tenn.
PETERSEN, W. E., Dairy Dept., Univ. Farm, St. Paul, Minn.
PHILLIPS, CLEMENT A., Univ. of Calif., Davis, Calif.
PIERCE, RAYMOND L., 24 Jaenicke Lane, Hamden, Conn.
PIKE, ADNA B., Dept. of Agri., Concord, N. H.

PLUM, MOGENS, Vetre Boulevard 51, Copenhagen, V, Denmark.
POE, CHARLES F., c/o J. F. Field, University of Colorado, Boulder, Colo.
POTTS, ARTHUR E., University of Sask., Saskatoon, Sask., Canada.
POTTS, ROY C., Bureau of Agri. Economics, U. S. D. A., Washington, D. C.
POUNDS, HARRY E., R. F. D. 4, Massillon, Ohio.
POWELL, MILTON E., Div. of Agri. Biochemistry, University Farm, St. Paul, Minn.
POWERS, A. J., Borden's Farm Products Co. Inc., 90 Third Ave., Brooklyn, N. Y.
PRATT, AVERY D., Dairy Dept., Virginia Polytechnic Inst., Blacksburg, Va.
PRATT, E. B., Consulting Dairyman, State Hospital No. 1, Fulton, Mo.
PRATT, HAROLD T., Philadelphia Dairy Products Co., 4th and Poplar St., Philadelphia, Pa.
PRENTICE, E. PARMALEE, Mt. Hope, Williamstown, Mass.
PRETZ, O. L., 1920 McGee, Kansas City, Mo.
PRICE, WALTER V., Univ. of Wis., Madison, Wis.
PRICHETT, PAUL S., Mead Johnson & Co., Evansville, Ind.
PROUTY, CHAS. C., Wash. Agri. Expt. Sta., College of Agri., Pullman, Wash.
PRUCHA, MARTIN I., Dept. of Dairy Husb., Univ. of Ill., Urbana, Ill.
PULKRABEK, G. M., 608 S. Dearborn St., c/o Pure Milk Assn., Chicago, Ill.
PUTNAM, GEORGE W., 1127 Greenwood Ave., Wilmette, Ill.

Q

QUENCER, ALBERT B., 542 E. 19th St., New York, N. Y.
QUIGLEY, J. V., 5633 Troost Ave., Kansas City, Mo.
QUINN, J. D., P. O. Box 446, Water Valley, Miss.

R

RABILD, HELMER, Titusville Dairy Products Co., Titusville, Pa.
RAGSDALE, A. C., University of Missouri, Columbia, Mo.
RANGE, MANSEL, 16 Freeman Place, Needham, Mass.
RAVEN, H. C., c/o Raven Dairy Inc., 4th Avenue, Portland, Ore.
REAGOR, Dr. H. A., 30 Calif. Ave., Reno, Nevada.
REAVES, P. M., Virginia Polytechnic Inst., Blacksburg, Va.
REDDING, C. H., 160 Second St., Jackson, Mich.
REED, O. E., Bureau of Dairy Industry, U. S. D. A., Washington, D. C.
REED, PERCY E., Dept. of Agri., Regina, Sask., Canada.
REESE, CHAS. F., 2125 Third St. N. E., Washington, D. C.
REGAN, M. J., Dairy Dept., Columbia, Mo.
REGAN, W. M., Agri. Expt. Sta., Davis, Calif.
REICHLE, C. J., Borden's Farm Products Company of Michigan, 3600 Forest Ave. E. Detroit, Mich.
REICHART, E. L., Dept. of Dairy Husb., University of Nebraska, Lincoln, Nebr.
REID, WM. H. E., University of Missouri, Columbia, Mo.
REINEKE, E. P., Dept. of Dairy Husb., University of Missouri, Columbia, Mo.
RENNER, E. W., Dairy Husb. Div., College Station, Texas.
RENNER, K. M., Texas Technological College, Lubbock, Texas.
REYNOLDS, A. E., 5056 8th Ave., Sacramento, Calif.
REYNOLDS, FRANKLIN P., 723 S. State St., Salt Lake City, Utah.
RHODE, C. S., Dept. of Dairy Husb., College of Agri., Urbana, Ill.
RICE, FRANK E., Evaporated Milk Assn., 203 Wabash Ave., Chicago, Ill.
RICHRADS, WM. I., 2419 4th Ave., Calgary, Alberta, Canada.
RICHARDSON, DONALD, R. F. D. 8, Box 105, Akron, Ohio.
RICHARDSON, GEO. A., University Farm, Davis, Calif.

RICHIE, H. B., Chemical Lab., Swift & Co., Union Stock Yards, Chicago, Ill.
RIDDELL, W. H., Dept. of Dairy Husb., College of Agri., Manhattan, Kans.
RISHOI, A. H., Dairy Industry Bldg., Cornell Univ., Ithaca, N. Y.
RIVE, HENRY, Dairy Commissioner, Dept. of Agri., Victoria, B. C., Canada.
ROADHOUSE, C. L., University of Calif., Davis, Calif.
ROBERTS, GEORGE V., Milton, Vt.
ROBERTS, RALPH E., Dept. of Dairy Husb., Agri. Expt. Sta., Lafayette, Ind.
ROBERTSON, A. H., Director of State Food Lab., New York State Dept. of Agri., and Markets, State Office Bldg., Albany, N. Y.
ROGERS, DOUGLAS, Chief, Chemist, General Milk Co. Inc., Schonnhove, Holland.
ROGERS, L. A., Bureau of Dairy Industry, U. S. D. A., Washington, D. C.
ROGERS, LEONARD, 804 Temple Ave., Knoxville, Tenn.
ROSS, H. E., Cornell Univ., Ithaca, N. Y.
ROUNDY, ZOLA DOYLE, Hiram Smith Hall, Univ. of Wisc., Madison, Wisc.
ROYER, K. M., c/o Pure Milk Association, 608 S. Dearborn St., Chicago, Ill.
RUDNICK, A. W., Iowa State College, Ames, Iowa.
RUEHE, H. A., Dairy Dept., College of Agri., Urbana, Ill.
RUFFER, R. H., Dept. of Animal Husb., State College Sta., Raleigh, N. C.
RUPEL, I. W., Agricultural Hall, College of Agri., Madison, Wis.
RUSSELL, JAMES, Kitsap Dairymen's Assn., 801 Park Ave., Bremerton, Wash.
RYAN, PARKER, 44 Pinkham Road, Medford Hillside, Mass.

S

SALISBURY, GLENN W., Dept. of Animal Husb., College of Agri., Ithaca, N. Y.
SALISBURY, S. M., Dept. of Animal Husb., Ohio State Univ., Columbus, Ohio.
SALZBERG, H. K., Research Chemist Mellon Inst., Thackeray Ave. & O'Hara St., Pittsburgh, Pa.
SANDERS, GEORGE P., Bureau of Dairy Industry, U. S. D. A., Washington, D. C.
SARTORI, J. HAYDN, P. O. Box 835, Tulare, Calif.
SCHAEFER, O. G., Room 2304, 420 Lexington Ave., New York, N. Y.
SCHEIDENHELM, E. C., Dairy Extension, Dairy Bldg., University of Nebraska, Lincoln, Nebr.
SCHELLENGER, KERN K., R. F. D. No. 1, Jackson, Ohio.
SCHMEITER, ROY, U. S. D. A., Food & Drug Adm., Washington, D. C.
SCHROEDER, HERMAN C., 5738 Kenwood Ave., Chicago, Ill.
SCOTT, H. T., 2242 West Lawn Ave., Madison, Wis.
SEARING, LYALL D., 711 S. E. 11th Ave., Portland, Ore.
SEARLES, H. R., Agr. Ext. Division, University of Minnesota, Univ. Farm, St. Paul, Minn.
SEATH, DWIGHT M., Extension Div., Kansas State College, Manhattan, Kansas.
SEFF, IRVING, 571 South Fifth St., Columbus, Ohio.
SEIDEL, MARTIN, 403 E. John St., Bay City, Mich.
SELBY, HOWARD W., 88 Cambridge St., Charleston, Mass.
SEYMOUR-JONES, DR. FRANK L., c/o Borden Co., 350 Madison Ave., New York, N. Y.
SHARP, PAUL F., Cornell Univ., Ithaca, N. Y.
SHAW, ALFRED O., Penn. State College, State College, Pa.
SHAW, D. D., 1520 Wilmette Ave., Wilmette, Ill.
SHEPARDSON, C. N., Dept. of Dairy Husb., Texas A. and M. College, College Station, Texas.
SHERE, LEWIS, 53 W. Jackson Blvd., The Diversity Corp., Chicago, Ill.
SHERMAN, J. M., Dairy Industry Bldg., Ithaca, N. Y.

SHIMMONS, A. V., 2628 Imperial Ave., San Diego, Calif.
SHOVHOLT, DR. OSCAR, Biochemistry Div., University Farm, St. Paul, Minn.
SHRADER, Dr. J. H., Sealtest System Labs. Inc., 120 Broadway, New York, N. Y.
SHULTZ, EARL N., Extension Service, Iowa State College, Ames, Iowa.
SIDELMANN, I. O., State College, Pa.
SIMMONS, ROSELL CLAY, 1709 Sonoma Ave., Berkeley, Calif.
SIMONSON, OBEN J., State School and Home, Box 410, Bedfield, S. Dak.
SKINNER, H. C., Canadian Dairy & Ice Cream Journal, 122 Richmond St., West, Toronto 2, Ontario, Canada.
SLATTER, WALTER L., R. F. D. No. 4, Fremont, O.
SMART, PAUL H., Carnation Evap. Milk Co., Ocomowoc, Wis.
SMITH, A. I., Box 24, Knoxville, Tenn.
SMITH, F. E. A., Director Production, United Farmers' Coop. Cry. Assn., Morrisville, Vt.
SMITH, R. W., Dept. of Dairy Mfgs., Univ. of Vermont, Burlington, Vt.
SNOW, C. H., Snow & Palmer Branch, Beatrice Meadow Gold Dairies, Bloomington, Ill.
SNYDER, WILLIAM, 824 No. 8th St., Lebanon, Pa.
SOMMER, H. H., Dept. of Dairy Husb., Univ. of Wisconsin, Madison, Wis.
SØRENSEN, C. M., Dairy Division, Purdue Univ., Lafayette, Ind.
SØRENSON, E. M., 1200 James St., Chehalis, Wash.
SØRENSEN, M., Pelican Rapids, Minn.
SOURAPAS, GEORGE, c/o National Ice Cream Co., 2747 W. Harrison St., Chicago, Ill.
SOUTHWELL, B. L., Georgia Coastal Plain Expt. Sta., Tifton, Ga.
SPECH, W. A., 617 Union St., Millensburg, Pa.
SPENCE, BYRON, 9015 W. Maple St., Milwaukee, Wisc.
SPICER, DELMAR W., 80 East Lane Ave., Columbus, Ohio.
SPITZER, GEORGE, 1000 Stadium Ave., West Lafayette, Ind.
SPRAGUE, WM. HERBERT, c/o Adohr Milk Farms, Tulare, Calif.
SPROULE, W. H., Dairy Dept., Ontario Agri. College, Guelph, Canada.
STARK, C. N., Dairy Bldg., Cornell Univ., Ithaca, N. Y.
STEBNITZ, V. C., 102 Dairy Annex, Univ. of Wisconsin, Madison, Wis.
STEPHENS, DAN V., Fremont, Nebr.
STEWART, G. F., c/o Omaha Cold Storage Co., Omaha, Nebr.
STOLTZ, R. B., Dairy Dept., Ohio State Univ., Columbus, Ohio.
STORVICK, R. O., Mgr., Iowa State Brand Creameries, Inc., 1927 2nd St. S. W., Mason City, Iowa.
STOUT, LOGAN, 6132 Lebanon Ave., Phila., Pa.
STOWE, J. P., Box 189, Greenville, N. C.
STRACHAN, ROBERT C., 20605 Beechwood Drive, Cleveland, Ohio.
STRUBLE, EARLE B., 319 Washington Blvd., Kenmore, N. Y.
SUNFORS, E. F., 517 B. Wentworth Ave., Santa Barbara, Calif.
SUPPLEE, G. C., Research Lab., Dry Milk Co., Bainbridge, N. Y.
SUTERMEISTER, L. A., The Red Wing Corp., Dayton, Ohio.
SUTTON, T. S., Dept. of Animal Husb., Ohio State Univ., Columbus, Ohio.
SWALLEN, WILBUR, R. D. No. 1, Alliance, Ohio.
SWANNER, ROY O., Bureau of Milk Sanitation, State Dept. of Health, Albany, N. Y.
SWANSON, WILLIAM F., Youngstown Sanitary Milk Co., Youngstown, Ohio.
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SWIFT, H. L., Produce Operating Dept., U. S. Yards, Chicago, Ill.
SWOPE, W. D., Dept. of Dairy Husb., Penna. State College, State College, Pa.

T

TAUB, JOEL, 40 E. 17th St., Brooklyn, N. Y.

TAYLOR, GEORGE E., Dairy Husb. Dept., Michigan State College, East Lansing, Mich.
TEMPLETON, HUGH L., Hiram Smith Hall, College of Agri., Madison, Wis.
THEOPHILUS, D. R., Dept. of Dairy Husb., Moscow, Idaho.
THOMPSON, E. C., Bordens Milk Products Co., 15 Renwick St., New York, N. Y.
THOMPSON, E. C., 1792 Elmore Place, Brooklyn, N. Y.
THOMSEN, L. C., 1527 Jefferson St., Madison, Wis.
THOMSON, A. C., Farm Bureau Office, Washington, Kansas.
THORNTON, H. R., Dept. of Dairying, Univ. of Alberta, Edmonton, Alberta, Canada.
THURSTON, L. M., Dept. of Dairy Husb., College of Agri., W. Va. Univ., Morgantown, West Virginia.
TITUS, R. W., Control Lab., Nestles Milk Products, Inc., Marysville, Ohio.
TOBEY, JAMES A., Director of Health Service, The Borden Co., 350 Madison Ave., New York, N. Y.
TORGESON, H. E., 3870 17th St., San Francisco, Calif.
TRACY, PAUL H., Dept. of Dairy Husb., Univ. of Illinois, Urbana, Ill.
TRAVER, ORVIN A., R. F. D. No. 1, Box 2, Millbury, Ohio.
TRELOGAN, HARRY C., Div. of Dairy Husb., Univ. of Minn., University Farm, St. Paul, Minn.
TRENCH, H. A., c/o I. N. Hagan Ice Cream Co., Uniontown, Pa.
TRETSVEN, J. O., Dairy Specialist, Mont. State College, Bozeman, Mont.
TROUT, G. MALCOLM, Dairy Husb. Dept., Mich. State College, East Lansing, Mich.
TRUE, N. F., Mead, Johnson & Co., Evansville, Ind.
TUCKER, HUBERT, North Jersey Expt. Sta., Sussex, N. J.
TUCKEY, STEWART L., 101 Dairy Mfrs. Bldg., University of Illinois, Urbana, Ill.
TURK, KENNETH L., Dept. of Animal Husb., Cornell Univ., Ithaca, N. Y.
TURNBOW, G. D., 1404 Franklin St., Oakland, Calif.
TURNER CHARLES W., Dept. of Dairy Husb., University of Missouri, Columbia, Mo.
TWINING, F. M., 198 N. Chancellor St., Newton, Pa.

U

USSELMAN, W., Galloway-West Co., Fon du Lac, Wis.
USTLER, GEO. H., c/o Angeles Coop. Cry. Port Angeles, Wash.

V

VACHA, G. A., Room 527 State Office Bldg., St. Paul, Minn.
VAN NORMAN, H. E., 5840 Stony Island Ave., Chicago, Ill.
VOGT, C. W., 166 E. 71st St., New York, N. Y.
VON LANGE, OSCAR, Soledad, Calif.
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W

WALLACE, E. T., Dairy Extension, Purdue University, Lafayette, Ind.
WALMSLEY, F. D., 177 N. Grove Ave., Oak Park, Ill.
WALTS, CHARLES C., P. O. Box 975, Parkersburg, W. Va.
WARD, FRED C., Nat. Dairy Products Corp. Inc., 1403 Eutaw Place, Baltimore, Md.
WARD, RUEL E., Dept. of Dairy Husb., State College Pa.
WARREN, T. R., 1734 N. E. Halsey Ave., Portland, Ore.
WATERSTREET, NEAL, Federal Trade Comm., Washington, D. C.
WATSON, PAUL A., 3255 Foothill Blvd., Pasadena, Calif.
WATT, A. L., Northern Great Plains Dairy Station, Mandan, N. D.
WEAVER, EARL, Okla. A. & M. College, Stillwater, Okla.

WEBB, BRYON, Bureau of Dairy Industry, U. S. D. A., Washington, D. C.
WEBNEY, HUGH W., 1002 Tacken St., Flint, Mich.
WEBSTER, C. F., Scudder Ranch, Box 36, Yakima, Wash.
WEBSTER, DUDLEY B., Box 955, Glenodale, Ariz.
WECKEL, KENNETH G., Univ. of Wisc. Dept. of Dairy Industry, Madison, Wis.
WEED, THEODORE H., 20 Beech St., Woodsville, N. H.
WEISER, HARRY H., Dept. of Bacteriology, Ohio State University, Columbus, Ohio.
WEISKIRCHER, RICHARD H., 300 N. 5th St., Martins Ferry, Ohio.
WEISSERT, A. D., c/o Schlosser Bros. Cry., Plymouth, Ind.
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WELCH, R. R., Dairy Ext. Specialist, Pa. State College, State College, Pa.
WETTLING, G. R., 163 West Avenue, Fairport, N. Y.
WHITAKER, R., National Dairy Products Corp., 1403 Eutaw Place, Baltimore, Md.
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WILBUR, J. W., Smith Hall, Purdue University, Lafayette, Ind.
WILHOITE, L. J., Southern Dairies, Inc., 457 Dodson Ave., Chattanooga, Tenn.
WILLARD, H. S., College of Agri., University of Wyoming, Laramie, Wyoming.
WILLIAMS, G. A., Purdue University, West Lafayette, Ind.
WILLIAMS, MARTIN L., J8., Edgemar Dairy, 346 Rose Ave., Venice, Calif.
WILLIAMS, SAMUEL B., R. D. No. 2, Middletown, Pa.
WILLMANN, JOSEPH, Derby, Conn.
WILSON, GEO. W., 1023 N. 49th St., Seattle, Wash.
WILSON, H. L., Bureau of Dairy Industry, U. S. D. A., Washington, D. C.
WILSON, LOGAN T., Walker-Gordon Lab. Co., Inc., Plainsboro, N. J.
WILSTER, G. H., Oregon State Agri. College, Corvallis, Ore.
WINKJER, JOEL G., Bureau of Dairy Industry, U. S. D. A., Washington, D. C.
WOODS, HEBER O., 2220 Oak Glen Place, Los Angeles, Calif.
WOODWARD, A. M., Castanea Dairy Co., Inc., 230 N. Broad St., Trenton, N. J.
WOODWARD, EDWIN G., Dairy & Food Commissioner, State Office Bldg., Hartford, Conn.
WOODWARD, T. E., Beltsville, Md.
WOOLMAN, HENRY N., Supplee Wills Jones Milk Co., 1523 North 26th St., Philadelphia, Pa.
WRIGHT, ERSKINE C., The Dairy Labs., 2615 Olive St., St. Louis, Mo.
WRIGHT, KENNETH E., 3 Spring St., Duluth, Minn.
WYLIE, C. E., Dairy Dept., Univ. of Tennessee, Knoxville, Tenn.
WYENO, JURO, S. M. A. Corp., Mason, Mich.

Y

YALE, M. W., N. Y. Agri. Expt. Sta., Geneva, N. Y.

Z

ZAKARISSEN, BEN, 2201 Kennedy St., Minneapolis, Minn.
ZAVAGALI, V., Laboratorio della Samita, Piazza Vittoris Emmanuelle 13, Rome, Italy.
ZIEGLER, PAUL D., 418 Hazle St., Tamaqua, Pa.

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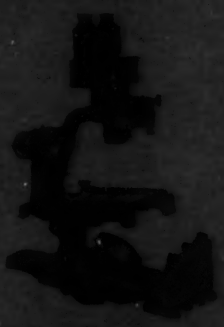

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